Impacts of freeze-thaw processes on phosphorus release from a variety of cover crop species in a temperate climate

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Cover crops (CC) are grown by farmers as an agricultural best management practice (BMP) to improve soil health during the non-growing season (NGS), and potentially reduce soil erosion. Some managers have cautioned against the use of CC due to their potential to act as sources of phosphorus (P) following exposure to freeze-thaw cycles (FTC), given that P has been strongly linked to eutrophication and algal blooms in freshwater systems. Existing literature on the effects of FTC on CC has indicated that the degree of P leaching may be influenced by multiple factors, including the number and/or magnitude of FTC, CC species, and the duration, intensity, and type of leaching event. However, much of the research has been done in laboratory studies, using conditions that are not representative of temperate climates or practices used in Southern Ontario. The objectives of this thesis were to evaluate the effects of FTC intensity and CC species on P release, using conditions and CC species commonly used in Ontario. These objectives were addressed in two separate experiments. The first experiment was done in the laboratory, where clippings of five CC species were exposed to five FTC at varying intensities (4 °C, -4 to 4 °C, -18 to 4 °C, and -18 to 10 °C), prior to extraction of P and nitrogen (N). The impact of termination was evaluated by comparing living CC samples to CC which were terminated with glyphosate. The second experiment was done in the field, and included seven plots of different CC species and mixtures. Plant and soil samples were analysed for P content throughout the NGS, and runoff and shallow groundwater were sampled on an event basis to evaluate the timing of P release from CC and the pathways through which the P moved. This thesis showed that CC species ranged greatly in P released after FTC, but N leaching was unaffected. Heavy frost (< -18 °C) increased P release, particularly from frost-intolerant species, whereas minimal FTC (> -4 °C) did not. Termination resulted in increased P and ammonium-N release from all species, but did not have an additive effect in combination with FTC. In a field setting, P release from CC was smaller in magnitude than was observed in the lab, consistent with the more moderate air temperatures that occurred. However, P release from CC was not related to P concentrations in surface soils and shallow groundwater, and, the pool of water extractable P was much smaller in the CC than in surface soil (5 cm). This thesis has shown that the tested CC species did not present risk for increased edge of field P loss, which is predicted to be consistent with other CC species grown in temperate climates. Planting CC should continue to be promoted as a BMP in Southern Ontario; however, in regions with colder climates, frost-tolerant CC species are recommended.

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Chapter 1- Introduction and Problem Statement

The eutrophication of freshwater systems through excess phosphorus (P) input has been well linked to algal blooms, because P is typically the limiting factor in algal growth (Smith et al., 1998; Schindler et al., 2012; Michalak et al., 2013; Jarvie et al., 2017). In marine environments, similar blooms are linked to nitrogen (N) limitation in algal communities, and management of N input from coastal areas remains a concern (Ryther and Dunstan, 1971; Gruber and Galloway, 2008; Congreves and Van Eerd, 2015). Algal blooms lead to a number of negative effects on an ecosystem, including: increased turbidity, hypoxia, and loss of biodiversity (Smith et al., 1998; Anderson et al., 2002). Human communities are also impacted by harmful algal blooms, which can result from algal decomposition impairing the recreational value of water-bodies, or the loss of potable water resulting from toxic cyanobacteria (Smith et al., 2015; Jetoo et al., 2015). The eutrophication of Lake Erie and many of its tributaries is one of the most pressing issues faced by the region; a large source of P in the watershed comes from agricultural land use, which is prominent in both the United States and Ontario (Michalak et al., 2013; Jarvie et al., 2017).

Agricultural best management practices (BMPs) are used by farmers to mitigate the loss of P; however, the dynamics of different forms of phosphorus, *i.e.* particulate vs dissolved fractions, may be impacted differently by each BMP (Sharpley and Smith, 1991; Sharpley et al., 1994; Rittenberg et al., 2015; Bergstrom et al., 2015). Cover crops (CC) are plants grown not for harvest or profit, but for a variety of benefits for soil health and erosion reduction, and are left on fields during the autumn and winter months, referred to as the non-growing season (NGS; Dabney et al., 2001; Blanco-Canqui et al., 2015). While CC are used to reduce the loss of particulate P (PP) through soil erosion, potential has been demonstrated for CC to act as a source of dissolved reactive P (DRP) when exposed to freeze-thaw cycle (FTC) conditions (Sharpley and Smith, 1991; Bechmann et al., 2005). The damage to plant cells caused by repeated FTC ruptures cell walls, and enables nutrient leaching into water that contacts CC tissues

(Tukey and Morgan, 1963; Burke et al., 1976). Other nutrients are variably susceptible to leaching from plant tissues, for example, N and potassium (K) are more readily leached than P (Chapin, 1980); however, research has shown FTC has less impact on N leaching compared to P (Miller et al., 1994). The degree of P release has been shown to be affected by numerous factors, including the number of FTC, the magnitude of FTC, the species of CC, and the method of extraction (Bechmann et al., 2005; Liu et al., 2013; Øgaard, 2015; Lozier and Macrae, 2017). Most studies have demonstrated potential P release under extremely cold conditions, representative of regions such as the plains of Western Canada or Scandinavia, using temperatures and practices common to those areas (Elliot, 2013; Liu et al., 2014). Uncertainty remains in how FTC may impact CC P release in more moderate climates, such as Southern Ontario, following management practices typical for this region. The objective of this thesis was to evaluate the impact different factors may have on P loss from CC under conditions typical of cool, temperate climates.

Chapter 2- Review of Literature

2.1 Terrestrial phosphorus dynamics

2.1.1 The phosphorus cycle

Phosphorus is a mineral nutrient required by all living things for cellular growth; as such, the P cycle involves both biological and geochemical reactions (Chapin, 1980; Smeck, 1985). As a non-volatile compound, P moves throughout the environment as either PP or in smaller compounds as DRP, which may be either organic or inorganic (Spivakov et al., 1999; Haygarth and Sharpley, 2000). Forms of SRP typically exist in the environment as orthophosphoric acid or orthophosphate; speciation is driven by soil pH (Holtan et al., 1988). Phosphorus enters terrestrial soils through the uplifting and breakdown of apatite bedrock material, the primary P mineral (Sharpley, 1995). As primary minerals are broken down into smaller sizes, the P is transitioned into the pool of DRP. The pool of DRP is readily bound into other mineral forms, or absorbed into soil biota (Smeck, 1985). The concentration of inorganic P is typically greater than organic P, ranging from 50-90% of the TP content; however, soils are extremely variable, and inorganic P levels have been found as low as 10% (Sharpley, 1995).

The binding of DRP to soil sediments leads to the formation of secondary P minerals. The initial sorption is a quick process which leaves the newly formed compound moderately labile, such that it may readily return to the DRP pool (Edzwald et al., 1976). Over time, the bound P will be drawn further into sediment particles, and held more strongly (Sharpkey, 1995). Phosphorus is bound loosely within hours, whereas the formation of secondary P minerals can take weeks, and the full occlusion of P may take hundreds of years (Smeck, 1985). The primary form of P binding is adsorption with iron and aluminum oxides, which are positively charged, opposite the negatively charged orthophosphate ions; the availability of sorption sites is dependent on redox conditions, and soil pH may limit or improve the sorption capacity of a soil (Edzwald et al., 1976). In alkaline, calcareous soils, phosphate may displace carbonate from calcium carbonate and form a precipitate of calcium phosphate (Holtan et al., 1988;

Baifan and Yichu, 1989). Calcium precipitation may be a more important mode of action in calcareous soils; however, literature directly comparing the magnitude of the mechanisms is limited.

Biological P cycling begins with uptake from the soluble P pool; once absorbed by soil biota, P can continue to cycle within the microbial community or transition into plant tissues (Smeck, 1985). The residence time of P held in decomposing organic material can range depending on the molecular stability; for example, P held in humic acids is bound more strongly than in phospholipids (Sharpley, 1995). In temperate areas, the temporal dynamics of biological P cycling are impacted by cold winter temperatures, which include the effects of plant dormancy, as well as the occurrence of freeze-thaw cycles (FTCs), which are discussed in detail in section 2.4.

2.1.2 Phosphorus in plants

The uptake and metabolism of P in plants is affected by many factors: plant species, availability of the nutrient, the mycorrhizal community, season, and climate (Morton, 1977; Bolan, 1991; Hobbie, 1992). The impact of species and environmental availability is closely linked, as species evolve to handle nutrient stress or surplus based on their environment (Chapin, 1980). Agricultural soils are typically kept higher in bioavailable P through regular fertilizer input which promotes greater yields than natural environments. Plant growth is often limited by nutrient requirements, primarily nitrogen, and in some situations phosphorus; though agricultural fertilization limits this effect (Herridge et al., 2008; Shen et al., 2011). As well, plant growth may also be limited by climate, water availability, or growing degree days in a season, depending on regional characteristics (Chapin et al., 1987). Because orthophosphate ions bind readily with soil, the movement of P to plant roots is incredibly slow and limits the diffusion rate across root membranes (Schachtman et al., 1998). Transport across cell membranes is an active process linked to hydrogen ion transport; H^+-ATPase pumps located within the membranes produce a charge gradient which enables the co-transport of P (Ullrich-Eberius et al., 1981). For many plant species, nutrient uptake rates are greatly benefited by mycorrhizal associations (Shen et al., 2011).

While mycorrhizae use the same P sources as plants, they have greater uptake rates; for example, the greater surface area for diffusion and utilizing a greater volume of soil enables more diffusion (Schachtman et al., 1998). As well, mycorrhizae can alter soil chemistry to desorb P from iron and aluminum oxides (Bolan, 1991).

When taken up into a plant, P is a highly mobile nutrient, similar to N and K, which allows for distribution via the xylem to areas of greater need, typically to areas of meristem growth (Chapin, 1980). When root P absorption is insufficient for new growth, P is translocated from older leaves to younger, developing leaves, which occurs through the phloem (Mimura, 1995). Within plant cells, P is contained within organic and inorganic compounds. Phosphorus is stored throughout plant tissue in the apoplast, the cytoplasm, vacuoles, and the nucleus—homeostasis is maintained between these pools through regular translocation (Mimura, 1995; Schachtman et al., 1998). Phosphorus is an important component in organic compounds, including nucleic acids and phospholipids, while inorganic P exists largely as adenosine triphosphate and orthophosphate ions (Chapin, 1980; Schachtman et al., 1998). The form of P may vary seasonally in some species, for example, some perennial species increase phospholipid content in response to winter temperatures, thereby decreasing the fraction of inorganic P (Chapin and Kedrowski, 1983).

All plants have some degree of nutrient leaching through semi-permeable tissues, which can result from rainfall, dew, snowmelt, or during ponding events, and affects all inorganic mineral nutrients and some organic compounds (Tukey, 1970). Compared to other nutrients, P is less available to leaching from plant tissue than N and K; however, many factors may increase the magnitude of nutrient loss, including damage to plant tissues (Tukey and Morgan, 1963; Chapin, 1980). The impacts of FTC on P leaching are discussed in detail in section 2.4.

2.1.3 Phosphorus transport pathways

Phosphorus is moved through the environment following the hydrological cycle, from terrestrial environments to aquatic environments, as orthophosphate ions (the predominant form of mineral P) and larger P compounds are not volatile and are typically predominantly transported by moving water (Holtan et al., 1988). The magnitude and form of P transported from agricultural systems is influenced by the hydrological transport pathway: overland flow or infiltration. Water contacting the soil surface will infiltrate into porous spaces between soil sediments, with exceptions occurring on frozen or fully saturated soils (Dingman, 2015). Infiltration rates may be affected by sediment type, presence of vegetation, antecedent moisture conditions, and macropore presence (Dunne et al., 1991; Sinaj et al., 2002; Beven and Germann, 2013; Dingman, 2015). Phosphorus which has infiltrated into the soil column has the greater potential to adsorb to sediments, as described in section 2.1.1; however, in sediments with high P content, net desorption may increase concentrations in infiltrate (Andersson et al., 2015). Flow through macropores may limit the contact time of P with sediment particles, enabling P movement to deeper depths, and if tile drains are present, may transport water and P directly to surface waters (Vogel et al., 2005; Bevan and Germann, 2013; Svanback et al., 2014). Macropore pathways are particularly important to consider in clay systems, as the shrink-swell dynamics may cause the formation of large, interconnected networks of cracks which move P to low depths quickly (Messing and Jarvis, 1993). As well, clay cracks regularly reform in the same space, resulting in limited capacity for P buffering as sediments lining a macropore reach sorption capacity (Kosmas et al., 1991).

Overland flow occurs when more water reaches the soil surface that does not infiltrate into the soil; which can occur as Hortonian overland flow, or Dunne overland flow (Dingman, 2015). Hortonian overland flow, also referred to as infiltration excess, is dependent on two factors: the magnitude of precipitation, and the surface characteristics of a hillslope (Fiedler and Ramirez, 2000). Infiltration excess overland flow results from high intensity storm events during which, precipitation occurs at a greater

rate than water can move into the soil matrix (Delfs et al., 2009). Dunne overland flow, also referred to as saturation excess, occurs when soil cannot penetrate into the soil column, which can be the result of a high water table, a shallow permeable layer, or frozen soils, and is more dependent on long duration storm events, or repeated storms in a short period of time (Dingman, 2015). In either case, once enough water has ponded to overcome the hydraulic resistance created by the roughness of the slope, water will begin to flow according to the topography of the landscape (Delfs et al., 2009; Dingman, 2015).

The transport of P through each of the pathways and mechanisms is highly dependent on the form of P available in a system. Phosphorus is typically considered to be found in two fractions, dissolved and particulate, based on what passes through or is retained by a 0.45 µm filter, respectively (De Haan et al., 1983; Haygarth and Sharpley, 2000). Filtration is an imperfect metric for comparing P fractionation, the soluble pool may contain P sorbed to fine particles as well as free forms of orthophosphate, which can differ in reactivity (Haygarth and Sharpley, 2000). These groupings can be split further into organic and inorganic compounds, which can further affect biogeochemical processes, particularly biological uptake (Spivakov et al., 1999).

Both dissolved reactive P (DRP) and particulate P (PP) may be transported by either pathway, overland flow or infiltration (Kronvang, 1992; Heathwaite and Dils, 2000). When moved into the soil matrix, DRP is considered reasonably immobile, as it is quickly sorbed to soil sediments or taken up by the biological community (Edzwald et al., 1976; Ruttenberg, 2003). Indeed, great potential for P loss occurs when P is transported via overland flow, or through macropores directly to tile drains, and therefore, most P loss from agricultural systems occurs during periods of snow melt or during large storm events (Pionke et al., 1996; Heathwaite and Dils, 2000; Jamieson et al., 2003; Van Esbroeck et al., 2017). The loss of PP in overland flow has been shown to account for up to 90% of total P (TP) in an agricultural watershed (McDowell et al., 2001), and even if only limited overland flow occurs, it may still

contribute the majority of the annual P export (Jamieson et al., 2003). Mellander et al. (2015) suggest that the ration of precipitation to runoff may be the best predictor of P export in overland flow, and that events with little runoff are ineffective at transporting PP, with the greatest losses occurring with peak overland flow.

As well, subsurface transport of P through macropores has been shown to contribute a substantial portion of P export in tile drained fields. Jamieson et al. (2003) determined subsurface loss during snow melt accounted for over a third of TP exported from an agricultural field. Macropores as small as 1 mm in diameter have been found to move P through a soil column with minimal loss (Geohring et al., 2001). Total P concentrations of up to 1.2 mg/L have been observed passing through macropores to depths of 15 cm (Heathwaite and Dils, 2000). Subsurface loss varies between sediments. Despite the greater potential for P sorption in clay soils, deep crack formation can result in more P loss than sandy soils, which have greater rates of matrix flow (Andersson et al., 2013).

2.2 Nitrogen dynamics

The terrestrial N cycle differs substantially from P, as gaseous forms of N, primarily N_2, regularly cycle between the atmosphere, soil, and biological matter (Gruber and Galloway, 2008). However, N_2 is unavailable to plants and must be fixed by nitrogen fixing bacteria before assimilation can occur (Stewart, 1967; Howarth et al., 1988). Nitrogen is fixed into the form of ammonium, NH_3^+, which is also the form produced by bacterial mineralization of detritus and can be taken up by plants (Verstraete and Focht, 1977; Hart et al., 1994). Nitrogen fixation occurs in limited groups of bacteria, some associated with rhizobial nodules of legumes, while others live freely in soil, as well as the aquatic cyanobacteria (Stewart, 1967; Howarth et al., 1988). As a positive ion, ammonium is readily bound to soil particles, and is not considered at risk for leaching from terrestrial systems (Nommik and Vahtras, 1982). Nitrification is the process of oxidizing ammonium into nitrite, NO_2^-, and nitrate, NO_3^-, which may be taken up into plants or undergo denitrification back to nitrogen gas (Verstraete and Focht, 1977; Hart et al., 1994). As

a component of all amino acids, N is required for protein synthesis and is a critical part of various cellular functions in plants (Osborne et al., 1908). As an anion, nitrate is less readily sorbed to sediments than ammonium, and may be exported by runoff (Quemada et al., 2013). Because N is typically the limiting nutrient in marine environments, the management of nitrate export is critical for coastal regions to prevent nuisance algal blooms (Ryther and Dunstan, 1971). Nitrogen is also commonly a growth limiting nutrient in terrestrial systems, and in agricultural systems, farmers must work to balance the fertilization needs of a crop and the potential for export (Chapin, 1980; Quemada et al., 2013)—agricultural best management practices (BMPs) are discussed in sections 2.3 and 2.4, with a focus on CC. Cover crops are used to manage N over the NGS and have been shown to reduce N export (O'Reilly et al., 2012; Blanco-Canqui et al., 2015). Nitrogen is fairly susceptible to leaching from plant cells, including CC; however, leaching has not been found to increase in relation to FTC which is of concern for P, discussed in section 2.5 (Chapin, 1980; Miller, 1994).

2.3 Phosphorus management in agriculture

Managing P export in agricultural systems involves balancing many factors, which may work in opposition with one another (Jarvie et al., 2017). For example, farmers may utilize the BMP of no-till to mitigate overland flow P export; however, reducing tillage enables macropore formation, which can increase subsurface loss, discussed in section 2.1.3. (Geohring et al., 2001; Vogel et al., 2005). As well, farmers must consider factors such as equipment cost, working hours, and potential reduction in crop yield from development and maintenance of new BMPs (Myers and Watts, 2015). The primary concern for P management is maintaining optimal concentrations of plant available P, which requires regular P input through fertilization, as P is regularly removed from agricultural systems through crop harvest in addition to normal P cycling loss. Current BMPs for P management target both the supply and transport of P, and may include: the 4r system, conservation or no-till, controlled tile drainage, cover crops (CC), and riparian buffers (Barling and Moore, 1994; Sharpley et al., 1994; Gyssels and Poesen, 2003; Ryder

and Fares, 2008; Strock et al., 2010; Tan and Zhang, 2011; De Baets et al., 2011; Shipitalo et al., 2013; Johnston and Bruulsema, 2014; Rittenburg et al., 2015; Kleinman et al., 2015). The effect of any one BMP on P export is dependent on localized soil and climate factors, and BMPs are best used in conjunction for optimal P management (Knapen et al., 2007; Lam et al., 2016). Only CC are discussed in detail in this literature review.

2.3.1 Soil test phosphorus

To prevent P from becoming growth limiting, farmers monitor soil P concentrations to maintain optimal concentrations (OMAFRA, 2017). As different fractions of P occur in soils with variable lability (discussed in section 2.1.1), the quantity of P extracted from a soil sample is dependent on the strength of the extractant (Wang et al., 2013). For example, extractions done using deionized water, sodium bicarbonate, and sulfuric acid result in increasingly higher concentrations of P, and used in a series of sequential extractions, the specific fractionation of P can be determined (Wang et al., 2013; Audette et al., 2016). In agricultural management, soil tests are used to quantify the fraction of P which is bioavailable, including soluble and weakly sorbed forms which can be taken up by plant roots and mycorrhizal fungi (Chapin, 1980; Schachtman et al., 1998; Carter and Gregorich, 2006). As P interacts differently in different soil compositions, a variety of metrics are available for determining soil test P, such as Olsen P, Mehlich-3, and Bray tests (Carter and Gregorich, 2006). Olsen P, a sodium-bicarbonate extraction, is recommended for use in systems with alkaline soils, where a greater proportion of P is held by calcium precipitation rather than by sorption to iron and aluminum oxides (Olsen et al., 1954). In Ontario, guidelines recommend maintaining soil test P at 12-18 mg/kg (Olsen P) to maximize crop yields while limiting the potential environmental impact (OMAFRA, 2017).

2.4 Cover cropping as an agricultural management practice

Cover crops are plants grown by farmers that are not intended for harvest or profit, and have been referred to in the literature by other names, including catch crops, surface crops, and green

manure, depending on the targeted benefit from growing CC (Kaspar and Singer, 2011). The suite of benefits offered by CC has been well demonstrated and thoroughly reviewed, and includes: reduction in soil erosion, weed suppression, increased soil organic matter, infiltration capacity, soil aggregate stability, reduced soil compaction, nutrient scavenging, and nitrogen addition (Blanco-Canqui et al., 2015; Kaspar and Singer, 2011; Dabney et al., 2001). Myers and Watts (2015) suggest that CC acreage of surveyed farmers in the United States roughly tripled between 2009 and 2015, and usage is predicted to continue increasing.

As well, the planting of CC mixtures has become increasingly more popular with CC growers (Myers and Watts, 2015), as planting multiple CC species is typically expected to provide a wider suite of benefits than a monoculture CC (Haynes, 1980; Blanco-Canqui et al., 2015). For example, nitrogen fixed in legume nodules may be transferred to non-legume species via the root network of the CC mixture (Giller et al., 1991). Mixtures of CC have also been found to impact primary productivity compared to monocultures of the same species; if several species which typically produce greater biomass are grown together, reliance on different ecological niches and potential synergistic relationships may result in greater yields than a monoculture (Wortman et al., 2012; Cardinale et al., 2017). As well, the greater biodiversity should improve the ecological resilience of a field, resulting in more stable production in poor conditions compared to fields which are less species rich (Tilman, 1996; Peterson et al., 1997). However, the efficacy of CC mixtures is still not well understood, and mixtures do not always produce desired benefits (Cardinale et al., 2011).

The erosion reducing properties of CC are the result of several factors: increased infiltration from CC root tissue (Geohring et al., 2001; Bevan and Germann, 2013), increased surfaced roughness to slow overland flow movement and trap sediments (Dunne et al., 1991; Ryder and Fares, 2008), and maintaining soil aggregate stability with root tissues (Gyssels and Poesen, 2003; De Baets et al., 2011). In

addition, the combination of these factors provides an added benefit of reducing PP export by holding P bound to sediments on the field (Kleinman et al., 2005). While the potential for CC to reduce PP is generally accepted, the potential for CC to increase the increased export of DRP in overland flow has also been demonstrated (Sharpley and Smith, 1991). The natural leaching of P, and other nutrients, from plants (Tukey and Morgan, 1963), detailed in section 2.1.2, may be affected by freeze-thaw cycling (FTC) which occurs in many temperate and cold regions, where CC are left on fields during the NGS. Without a CC present, the loss of DRP from agricultural soils occurs primarily from desorption of sediment bound P and leaching from crop residues; with a CC scavenging P from the entire root zone of the soil profile, a greater pool of P is made more available to leaching, and potentially runoff (Andersson et al., 2013; Elliot, 2013).

2.5 Impact of freeze-thaw cycling on cover cropping and nutrient dynamics

As water in plant cells refreezes, the potential for crystals to form is increased, potentially rupturing the cell membrane and wall, and lysing the cell, thereby increasing the availability of nutrients for leaching (Burke et al., 1976; Tukey and Morgan, 1963). The magnitude to which FTC affects plant cells and the resulting P release is influenced by a number of factors. Differences between plant species have been shown to affect P release (Miller et al., 1994; Liu et al., 2014; Lozier and Macrae, 2017). Liu et al. (2014) suggest that frost-tolerant plants and species with high woody content in tissues should have a smaller response to FTC. Oppositely, plants that typically winter-kill, such as oilseed radish, have been shown to release significantly more P after FTC (Miller et al., 1994; Liu et al., 2013; Liu et al., 2014; Øgaard, 2015). The number of FTC plants experience will also affect the magnitude of leaching. For example, Bechmann et al. (2005) found that repeated cycling increased P release, although the effect was limited after approximately six cycles. Lozier and Macrae (2017) found repetition did not always increase P release from all CC, but the effect may be related to species differences.

Figure 2.1 Various cover crop species (left to right: oat, cereal rye, oilseed radish, hairy vetch) before (top) and after (bottom) exposure to freeze-thaw cycling

The freezing temperature can also affect the magnitude of P release. Many laboratory studies have evaluated potential leaching at temperatures of -18 °C (Bechmann et al., 2005; Liu et al., 2013; Riddle and Bergstrom, 2013; Liu et al., 2014). While -18 °C may be effective for indicating potential for P leaching, and is representative of climate conditions in Nordic regions or the Canadian plains, it does not represent climate conditions in more temperate areas, such as Southern Ontario. Only a few studies have examined the impact of warmer FTC temperatures (-4 °C), and more research on this factor is needed to compare with results from harsher FTC experiments (Øgaard, 2015; Lozer and Macrae, 2017).

The termination of CC is a factor which has not been well assessed in relation to FTC. Many CC growers choose to terminate with herbicide, or by mowing or plowing over the crop (Myers and Watts, 2015). Cover crops that do not winter-kill may be terminated in either the autumn or the early spring. While spring termination provides the most benefit from a growing CC, it is more difficult for farmers to manage the timing between soils drying and planting. Lozier and Macrae (2017) observed great

concentrations of P released from a terminated red clover CC; the limited amount of literature does not indicate how other species may react to termination, and more research is needed in this area.

Many studies have demonstrated the potential for CC to act as a source of P in laboratory settings; while controlled laboratory conditions give strong insight into potential risk factors for P leaching, field studies which run the course of the NGS are needed to more accurately evaluate P leaching. Øgaard (2015) found that different species of CC exposed to FTC had peak P release at various points during the NGS, in response to different intensities of FTC. Lozier et al. (2017) and Sturite et al. (2007) both observed peak P loss from some CC species after spring snowmelt, despite FTC exposure in the autumn. Evaluating P leaching in a field setting also includes the potential for soil uptake of plant-released P. Robertson et al. (2007) found no effect of CC leaching on runoff P concentrations, as great natural variability in runoff P concentrations did not reflect any potential CC P loss. Lozier et al (2017) found that edge of field P concentrations were unaffected by CC P leaching, and suggest infiltration enabled the soil to capture the leached P. Indeed, even in laboratory studies with shallow (15 cm) soil columns, P concentrations in leachate have been significantly reduced compared to plant leachate which did not pass through soils (Bechman et al., 2005; Riddle and Bergstrom, 2013).

The effect of CC mixtures on P loss has not been well evaluated in the literature and is poorly understood. As some CC species continue to grow while others are frost-killed, planting mixtures of these CC may enable frost-tolerant species to uptake P released other plants. As well, it is unknown how the improved resilience of CC mixtures may alter the effect of FTC on species prone to P leaching; in some cases frost-intolerant species may overwinter better when planted in mixtures (Exner and Cruse, 1993).

Better understanding how different CC species may respond to FTC in climate conditions that reflect the NGS of Southern Ontario will enable those farmers to make more informed choices for CC management and environmental protection.

2.6 Thesis Objectives

1) To examine the effect of FTC magnitude on P and N leaching from CC

2) To examine the variability in P an N leaching in response to FTC from various CC species

3) To examine the effect of herbicide-induced termination prior to FTC on P and N leaching from CC

4) To evaluate how water extractable P and total P concentrations vary throughout the length of the NGS between different CC species

5) To examine P concentrations in soil, soil water (leachate) and shallow groundwater throughout the NGS and determine if changes in water chemistry coincide with changes in the vegetation P pool

6) To determine if the observed changes in plant and soil P for specific CC species differs when they are planted as monocultures or polycultures.

Objectives 1, 2, and 3 are addressed in "Nutrient release from living and terminated cover crops under variable freeze-thaw cycles" (Chapter 3 of this thesis), and objectives 4, 5, and 6 are addressed in "Winter phosphorus release from different cover crop species relative to soil, runoff, and shallow groundwater phosphorus content in a temperate region" (Chapter 4 of this thesis).

Chapter 3- Nutrient release from living and terminated cover crops under variable freeze-thaw cycles

3.1 Overview

Cover crops (CC) are planted into fields during the non-growing season as a best management practice (BMP) for agronomic and environmental benefits. However, freeze-thaw cycles (FTC) may increase the availability of water extractable P (WEP) from damaged plant tissues, leading some to question their efficacy as a nutrient BMP due to their potential to release P during snowmelt. The objectives of this study were to experimentally determine the influence of: (1) FTC magnitude (4 °C, -4 to 4 °C, -18 to 4 °C, and -18 to 10 °C), (2) CC species [cereal rye (*Secale cereale* L.), oilseed radish (*Raphanus sativus* L. var. *oleoferus* Metzg Stokes), red clover (*Trifolium pratense* L.), oat (*Avena sativa* L.), and hairy vetch (*Vicia villosa* Roth)], and (3) termination using herbicide on the magnitude of WEP, NH_4^+, and NO_3^- release. Shoot tissue clippings underwent five FTC followed by extraction. Large magnitude FTC from -18 to 4 and -18 to 10 °C (heavy frost) elevated WEP release, whereas the -4 to 4 °C (light frost) treatment did not. Responses varied with plant type, where frost-intolerant species released more WEP than frost-tolerant species. In contrast, NH_4^+, and NO_3^- release did not increase following FTC. Termination elevated WEP and NH_4^+ release across all temperature treatments. The use of CC as a nutrient BMP should be used with caution in some regions, but in areas with mild winter climates, growing frost tolerant species without termination may reduce the risk of P leaching from vegetation in winter and early spring.

3.2 Introduction

Elevated levels of P transported into aquatic ecosystems from agricultural watersheds have contributed to the eutrophication of surface water bodies in Canada as well as freshwater habitats around the world (Smith et al., 1998; Schindler et al., 2012; Michalak et al., 2013; Jarvie et al., 2017). In salt water environments, eutrophication has been linked to anthropogenic N inputs (Gruber and

Galloway, 2008; Congreves and Van Eerd, 2015). Eutrophication is problematic as it impacts both ecosystem and human health (Anderson et al., 2002), which reduces the recreational value of lakes as well as their potential use as drinking water sources (Smith et al., 1998, Smith et al., 2015). Consequently, there is significant pressure to reduce the export of P and N from surrounding watersheds (International Joint Commission, 2014). Although researchers and environmental managers have attempted to manage nutrient export for decades (Sharpley et al., 1994), the occurrence of large algal blooms has increased due to a combination of climate drivers as well as the large intensity of agricultural land use in surrounding watersheds (*e.g.* Michalak et al., 2013; Smith et al., 2015). There is also evidence that the elevated P loads from agricultural systems may be an unintended consequence of conservation practices (Jarvie et al., 2017). Thus, an improved understanding of the efficacy of best management practices (BMPs) is needed, and potential unintended consequences must be identified and quantified.

The use of CC is a conservation practice that is growing in popularity (Wayman et al., 2016; Statistics Canada, 2017). Cover crops are plants grown by farmers for their benefits to the soil, environment, and future crop yields (Snapp et al., 2005; Blanco-Canqui et al., 2015). Cover crops have the benefit of reducing particulate P losses associated with soil erosion; however, concern has been raised about their potential to release dissolved reactive P (Tukey, 1970; Sharpley et al., 1994; Sturite et al., 2007; Liu et al., 2014). Phosphorus loss through leaching is typically minor; however, certain conditions and mechanical processes have been found to increase the concentration of nutrients in leachate (Tukey and Morgan, 1963; Bechmann et al., 2005; Lozier and Macrae, 2017; Lozier et al., 2017). Of particular importance for northern temperate regions is the effect of freezing on P release from plants, as damage caused by FTCcan rupture cell walls, making significant amounts of nutrients available for export (Tukey and Morgan, 1963; Miller et al., 1994; Bechmann et al., 2005; Elliot, 2013). Other dissolved compounds such as N species may also be released through FTC processes (Sturite et al., 2007), although some have

suggested that N may be affected to a lesser degree than P by FTC damage (Miller et al., 1994). Thus, an improved understanding of the impacts of FTC on coupled P and N release is needed to improve nutrient BMPs.

To assess the potential for winter P and N losses from CC, several studies have employed laboratory experiments. Factors that have been shown to impact the rate of P leaching from CC include the degree of FTC temperatures (Øgaard, 2015), species differences (Elliot, 2013; Liu et al., 2014), and the number of FTC (Bechmann et al. 2005). An increased number of FTC increases P release from CC, although this appears to plateau over time (Bechmann et al., 2005) and is not observed for all species (Lozier et al., 2017). The magnitude of freezing has also been found to make a difference, as plants exposed to FTC at -10 °C released substantially less P than plant samples left outdoors and exposed to temperatures less than -20 °C (Øgaard, 2015). With the exception of Øgaard (2015) and Lozier and Macrae (2017), most laboratory studies have used FTC temperatures such as -18 to 18 °C, or -18 to 12 °C (*e.g.* Bechmann et al., 2005; Liu et al., 2013a; Riddle and Bergstrom, 2013; Liu et al., 2014). Although these conditions were representative of extreme shifts in the Nordic climate (Liu et al., 2013a) or winter conditions in colder regions such as the Northern Great Plains of North America, they may not be appropriate for assessing how CC will be affected in more temperate regions where such extreme temperature fluctuations are rare. Thus, a greater understanding of the impacts of FTC magnitude on nutrient release from CC is needed.

Differences between CC species have also been found to affect potential P and N release from tissue under similar conditions (Miller et al., 1994; Elliot, 2013; Lozier and Macrae., 2017). For example, chicory (*Cichorium intybus* L.) and phacelia (*Phacelia tanacetfolia* Benth.), CC commonly used in Sweden, have minimal losses, possibly related to greater woodiness content (Riddle and Bergstrom, 2013; Liu et al., 2014) whereas oilseed radish and other species susceptible to winter-killing typically leach greater

quantities of P once exposed to freezing temperatures (Liu et al., 2013a; Liu et al., 2014; Øgaard, 2015; Lozier and Macrae, 2017).

An additional factor that may impact nutrient release from CC is termination in autumn. While some species are killed by freezing temperatures, some survive through the winter, which can be difficult to manage the following spring when fields are drying, and, waiting for termination may shorten the growing season. Consequently, some farmers choose to terminate CC prior to the arrival of winter. This is a common practice in Ontario, Canada. Termination is typically done through the application of an herbicide, but may also be done through ploughing or mowing (Cherr et al., 2006; Blanco-Canqui et al., 2015). Research comparing CC to dead plant residues (left after cash crop harvest to cover the soil) has shown that living plant tissue releases significantly more dissolved P than dried residues (Elliot, 2013; Lozier et al. 2017). Lozier et al. (2017) reported that FTC did not enhance P release from red clover and suggested that this may have been due to the application of glyphosate (*N*-(phosphonomethyl)glycine) to the CC several days prior to the experiment. However, recently terminated CC may not desiccate immediately, and may still be potential P sources compared to dried residues left from the previous crop that have different potential P release (Elliot, 2013). An improved understanding of the impact of termination on P release from CC is needed, as well as information on how this compares to P release from CC that are frozen while living.

Much of the evidence of winter P release from CC has been conducted in regions with harsh winters (or under harsh simulated conditions), on CC species typically grown in these regions, and on plants that are not terminated prior to freezing. The use of CC as a BMP has been widely promoted in temperate regions to reduce nutrient loading during the non-growing season as this is the period during which most P and N losses occur (Macrae et al., 2007; Congreves and Van Eerd, 2015; Blanco-Canqui et al., 2015). Some advisors have encouraged farmers to stop terminating CC or reconsider CC usage. To

assess the potential for winter P and N loss from CC in the Great Lakes region of North America and to refine CC-specific nutrient BMPs, this study used a laboratory experiment (factorial design) to examine the individual and combined impacts of (a) FTC magnitude, (b) CC species and (c) termination on WEP, nitrate (NO_3^-) and ammonium (NH_4^+) release.

3.3 Materials and Methods

3.3.1 Collection of Field Samples

Five CC species were used in this study: cereal rye, oilseed radish, red clover, oat, and hairy vetch. Samples were collected from commercial winter wheat (*Triticum aestivum* L.) fields in Southwestern Ontario, where wheat and cover crops were planted and grown according to typical grower practices. Consequently, CC were collected from different fields. Red clover was frost seeded into wheat in March. All other CC were planted after wheat harvest in late July. Oat and oilseed radish were grown together in one field and in a different field, cereal rye and hairy vetch were grown in strips. However, both fields were managed by the same grower.

Only tissue growing above ground was collected, with the exception of oilseed radish, where the tap root was analysed separately from the shoot tissue. Cover crop samples were collected by clipping the whole shoot at the soil surface, except for oilseed radish tap roots, which were pulled out and cut at the crown separating shoot from root tissue. Living samples were collected on 21 and 22 Oct. 2015. In this manuscript, the use of the term “living” was applied to fresh vegetation samples that were collected while plants were alive, to be compared with plants that were “terminated” using herbicides. It should be noted that living vegetation samples were clipped and no longer connected to root systems at the time of extraction (and thus not actually living); however, samples were green and turgid at the time of extraction. Glyphosate was applied to fields after the first sample collection and terminated CC samples were collected approximately two weeks later. Cereal rye and hairy vetch were sampled 13 days after application on November 5, 2015, red clover after 14 days on November 11, 2015, and oat and oilseed

radish after 15 days on November 11, 2015. All sprayed CC samples had senesced by the second collection period. Once collected, samples were bagged, stored in a cooler for transportation to the lab. A portion of each sample was immediately extracted (control treatment), while the bulk of the samples were separated into treatment groups. Oilseed radish roots were wet-wiped to remove all excess dirt, and all other samples were left untouched from the field. A small amount of rainfall occurred over the two-week period between the collection of "living" and "terminated" samples that may have leached some nutrients, and, there was a light frost. The coldest temperature recorded was -3 °C (Environment Canada, 2015b). In anticipation of this potential, a portion of the cover crops was left unsprayed in fields, from which a second round of unsprayed samples were collected at the same time as the terminated samples. Compared to samples collected during the first round, no significant difference was found between WEP concentrations in leachate, nor TP concentrations in plant tissues ($p > 0.05$; data not shown), indicating minimal leaching of P between the sampling periods.

3.3.2 Experimental Treatments

The treatment groups were developed with a full factorial design, such that all CC tissue samples from both living and dead plants, from every species underwent all of the different FTC. The FTC regimes used were: a control extracted immediately upon collection with no FTC, no freezing (held at 4 °C), -4 to 4 °C, -18 to 4 °C, and -18 to 10 °C. Samples were kept in freezing conditions for 12 hr, before moving to thawing conditions for another 12 hr to complete one cycle. During the course of the FTC, samples were kept in thinly packed, large ziplock bags, such that samples were evenly exposed to FTC temperatures, and no insulating effect occurred in the middle. All samples underwent five cycles, as Bechmann et al. (2005) suggest that available P levels were not significantly increased by further freezing events. The freezing temperature of -18 °C used in the current study is extreme, as is the frequency of FTC. However, this temperature and the FTC pattern used were chosen to maintain consistency with the experimental methods used in previous studies (e.g. Bechmann et al., 2005; Liu et al., 2013a; Riddle and Bergstrom,

2013; Liu et al., 2014) for the purpose of contrasting the impact of lighter FTC in the Great Lakes Region. After the final FTC, all samples were transferred to a 4 °C refrigerator prior to being extracted for the determination of WEP, done within 24 hr of the final thaw. A portion (approximately 15 g field moist) of each sample was dried at 80 °C for 24 h to determine moisture content to allow nutrient contents to be expressed per dry weight mass.

3.3.3 Sample Processing and Laboratory Analyses

Three replicates were sampled from each treatment group. Approximately 5 g (field moist) of shoot tissue was clipped to lengths of 4 cm or less (to fit within the 100 mL polyethylene extraction containers) after exposure to FTC. Tissue samples were batched to randomly take material from approximately 10-20 of different individual plants. Oilseed radish roots were sampled as individual pieces of root, taken as ~1 cm long cylinders from the midsection of the root. Extractions for WEP and N were conducted with 50 mL of deionized water for WEP, or 2M KCl for N. Samples were shaken for 1 hr to ensure thorough contact with plant material per Roberson et al. (2007). After shaking, leachate was immediately gravity filtered through Whatman No.42 filter paper until a minimum of 15 mL of filtrate accumulated.

Filtered extracts were immediately frozen and stored at -18 °C until analysis (~1 month). Several hours prior to measurements samples were fully thawed in a cool water bath (~12 °C). All sample analyses were done at the Biogeochemistry Lab at the University of Waterloo. Ortho-phosphate concentrations were determined using ion chromatography (DIONEX ICS 3000 equipped with IonPac AS18 analytical column). Nitrogen analyses were conducted using colorimetric techniques (Bran Luebbe AA3, Seal Analytical, Seattle, USA, NH_4^+: Alkaline salicylate with hypochlorite and nitroprusside, Method No. G-102-93; NO_3^-+NO_2^-: Alkaline hydrazine sulphate with copper, Method No. G-109-94). All analysis included 5% duplicates which fell within 5% of reported concentrations. All concentrations were presented as μg nutrient g^{-1} dry plant mass.

Plant samples analysed for TP content were oven dried at 100°C, and ground before digestion. Between 0.2-0.4 g of each tissue sample underwent a sulfuric acid/ lithium sulfate/ selenium digestion (Parkinson and Allen, 1975). Phosphorus concentrations were analysed with ammonium molybdate/ascorbic acid colorimetric methods [Methods G-103-93 (DRP), G-188- 235 097 (TDP, TKP) on a Bran-Luebbe Auto Analyzer III system, Seal Analytical, Mequon WI].

3.3.4 Determination of Biomass Estimates and Vegetation Nutrient Pools

To convert nutrient release values from CC tissues to approximate potential field-scale P pools, estimates of dry plant biomass (kg ha^{-1}) were made. Several of the species of plant samples were collected from fields containing a mixture of multiple CC species planted after winter wheat, thus determining whole field biomass was not possible. Sample values for cereal rye, oat, hairy vetch, and oilseed radish (shoot and root tissue were separated; only protruding root tissue was sampled) biomass were taken from a parallel, field-scale CC experiment planted after winter wheat in August 2016, and collected from three 0.25 m^2 quadrats in October 2016. The estimate of biomass value of red clover was a visual estimate by former provincial cereal specialist (pers. comm. Peter Johnson) determined when he collected the sample in October 2015 before glyphosate application.

3.3.5 Statistical Analyses

Analysis of results was done using IBM SPSS Statistics, version 21. A three-way ANOVA of CC species, FTC magnitude and life state factors was analyzed on each nutrient separately. Due to multiple significant two- and three-way interactions ($P < 0.05$), a series of two-way and one-way ANOVAs were conducted to determine how each factor influenced nutrient availability, corresponding with the study objectives. When a one-sample Kolmogorov-Smirnov test indicated a non-normal distribution, the data were transformed with the natural log. When the log-transformed data remained non-normal, the data were ranked, and analysed via non-parametric ANOVAs. With a normal distribution, parametric ANOVAs were run, with the Bonferroni correction applied for post-hoc analysis. When significant interactions

were found between two factors, the dataset was split by the levels of one factor, chosen based on the study objectives. For ranked datasets, the Scheirer-Ray-Hare test was used to examine the effects of two factors. When significant interactions existed between the two factors, the Kruskal-Wallis test was used applied to both factors. When the Kruskal-Wallis tests indicated significant differences, the Mann-Whitney U test was applied to each category. An alpha value of 0.05 was applied for all statistical testing.

3.4 Results

3.4.1 Effects of Temperature on Extractable Phosphorus and Nitrogen Concentrations in Living Vegetation

Freeze-thaw cycles increased WEP release from living vegetation; however, the magnitude of release differed with FTC magnitude and CC species (Figure 3.1a). The large magnitude FTC (heavy frost) increased WEP concentrations from all species, but differences in the magnitude of increase resulted in a significant interaction between FTC magnitude and CC species ($F = 2.987$, $p \leq 0.001$, Table 3.1a). A series of one-way ANOVA tests done for individual species reported a significant effect of FTC on WEP release for all species (Table 3.1b). The FTC treatments readily split into two distinct categories: light frost (4 °C and -4 to 4 °C) and heavy frost (-18 to 4 °C and -18 to 10 °C) (Figure 3.1a). All plant tissues frozen at -18 °C exhibited increased WEP availability, with WEP concentrations increasing several orders of magnitude on average. A series of t-tests found no significant differences in WEP between control samples (immediately extracted), those that remained unfrozen (held at 4 °C for five days) or those that underwent FTC at -4 to 4 °C. Similarly, plants that underwent FTC at -18 to 4 °C and -18 to 10 °C also had no significant difference in WEP.

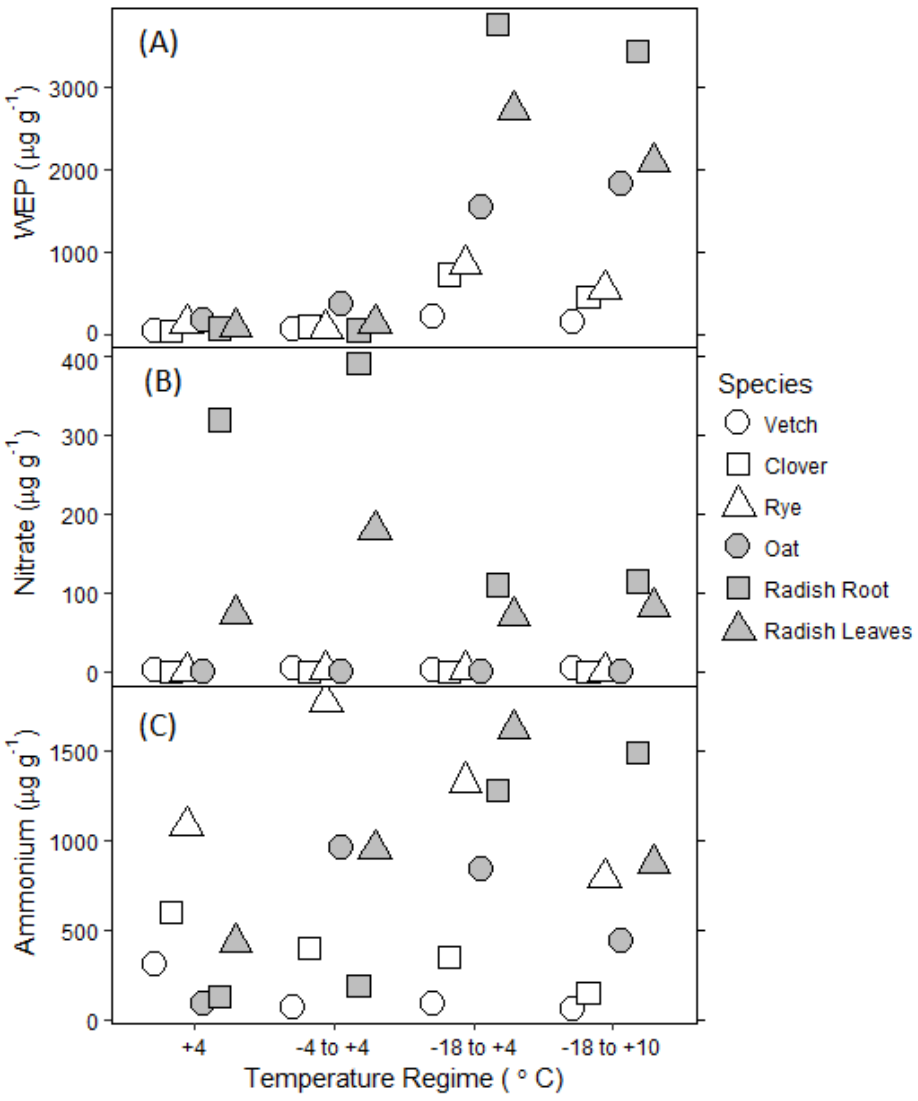

Figure 3.1 Nutrient [(A) water-extractable P (WEP), (B) nitrate-N, and (C) ammonium-N)] content extracted from cover crop tissues, without termination, after exposure to various freeze-thaw cycle magnitudes, split by cover crop species. Points indicate median nutrient content; variation between replicates was omitted for clarity.

In living tissue, the impact of FTC magnitude on N release did not follow a similar trend compared to WEP release (Figure 3.1b,c). Concentrations of extracted NH_4-N were highly variable with significant interaction occurring between FTC magnitude and CC species ($F = 9.445$, $p \leq 0.001$, Table 3.1a). When samples were split by species, a series of one-way ANOVA tests found significant effects of

FTC for most species (Table 1b); however, no consistent trends were observed between FTC treatments (Figure 3.1c). Compared to WEP and NH_4-N, very little NO_3-N (<400 μg g^{-1}) was extracted in any of the sample treatments (Figure 3.1b). No significant effect of FTC magnitude or interaction between FTC magnitude and CC species was found (Table 3.1a). Oilseed radish shoot and root tissue tended to have greater NO_3-N release than the other CC, which was not consistent with WEP nor NH_4-N released from the 4 °C and -4 to 4 °C (light frost) treatments.

3.4.2 Impacts of Termination on Extractable Phosphorus and Nitrogen Concentrations

A two-way ANOVA testing the impacts of FTC magnitude and the effect of termination as factors found a significant interaction between the factors on WEP release ($F = 11.10$, $p \leq 0.001$, Table 3.1c); consequently, each level of FTC magnitude was tested separately (one-way ANOVA) to determine the impacts of termination (Table 3.1d). In terminated CC compared to living CC plants, WEP availability was significantly elevated in the 4 °C treatment ($F = 27.23$, $p \leq 0.001$) and -4 to 4 °C treatments ($F = 28.95$, $p \leq 0.001$), whereas termination had no significant effect on plants exposed to heavy frosts of -18 °C (Figure 2, Table 3.1d). Once the plant tissue was severely damaged (*i.e.,* exposure to -18 °C) the maximum level of WEP was made available, and further damage, whether through frost or herbicide, did not have any additive effects. The magnitude of FTC did not impact the variability of WEP in terminated CC, but WEP in living CC was more variable with heavy frosts than light frosts (Figure 3.2).

Table 3.1 Results of ANOVA tests on the effects of freeze-thaw cycle magnitude (FTC), cover crop species (CC), and life state (living, terminated) on water extractable P (WEP), nitrate-N, and ammonium-N release. Results found significant at the 0.05 level are **bolded**. Where significant interactions were found in a two-way ANOVA the main effects were not considered.

		Water-extractable P				NO_3-N				NH_4-N			
		SS	*Test Stat.*	*df*	*Sig.*	*SS*	*Test Stat.*	*df*	*Sig.*	*SS*	*Test Stat.*	*df*	*Sig.*
(a) 2-way ANOVA testing effects of FTC x CC	FTC	115	F= 73.5	4	**0.000**	430	H= 0.6	4	0.960	30	F= 40.4	4	**0.000**
	CC	37	F= 19.0	5	**0.000**	52004	H= 76.6	5	**0.000**	61	F= 65.6	5	**0.000**
	FTC X CC	23	F= 3.0	20	**0.001**	2948	H= 4.3	20	1.000	35	F= 9.5	20	**0.000**
(b) 1-way ANOVA testing effects of FTC †	Hairy Vetch	6	F= 7.5	4	**0.005**	—	—	—	—	188622	F=57.0	4	**0.000**
	Red Clover	23	F= 35.8	4	**0.000**	—	—	—	—	379601	F= 9.7	4	**0.002**
	Cereal Rye	13	F= 6.3	4	**0.009**	—	—	—	—	3	F= 3.0	4	0.070
	Oat	17	F= 21.3	4	**0.000**	—	—	—	—	1703699	F= 16.1	4	**0.000**
	Radish Shoot	34	F= 11.5	4	**0.001**	—	—	—	—	2799880	F=8.5	4	**0.003**
	Radish Root	46	F= 20.9	4	**0.000**	—	—	—	—	5958073	F= 39.6	4	**0.000**
(c) 2-way ANOVA testing effects of Life State x FTC	Life State	58	F= 40.2	1	**0.000**	0	H= 0.0	1	1.000	83	F= 49.5	1	**0.000**
	FTC	54	F= 9.5	4	**0.000**	526	H= 0.8	4	0.941	30	F= 4.5	4	**0.002**
	Life State x FTC	64	F= 11.1	4	**0.000**	1210	H= 1.8	4	0.775	6	F= 0.9	4	0.492
(d) 1-way ANOVA testing effects of Life State ‡	Control	41	F= 50.3	1	**0.000**	—	—	—	—	—	—	—	—
	4 °C	39	F= 27.2	1	**0.000**	—	—	—	—	—	—	—	—
	-4 to 4 °C	39	F= 29.0	1	**0.000**	—	—	—	—	—	—	—	—
	-18 to 4 °C	2	F= 0.9	1	0.351	—	—	—	—	—	—	—	—
	-18 to 10 °C	0	F= 0.3	1	0.609	—	—	—	—	—	—	—	—
(e) 1-way ANOVA testing effects of CC Species under various temperature treatments	-4 to 4 °C Live	196617	F= 2.86	5	0.063	167	F= 40.7	5	**0.000**	5046070	F= 12.9	5	**0.000**
	-18 to 4 °C Live	39624370	F= 13.98	5	**0.000**	130	F= 16.6	5	**0.000**	4863918	F= 11.9	5	**0.000**
	-4 to 4 °C Terminated	27266725	F= 59.67	5	**0.000**	68	F= 82.8	5	**0.000**	20692388	F= 13.8	5	**0.000**
	-18 to 4 °C Terminated	11371777	F= 1.83	5	0.182	107	F= 131.6	5	**0.000**	176013618	F= 21.2	5	**0.000**

† *one-way ANOVA tests for individual species were only run where significant interaction was found in the 2-way ANOVA in (a)*

‡ *one-way ANOVA tests for individual FTC magnitudes were only run where significant interaction was found in the 2-way ANOVA in (c)*

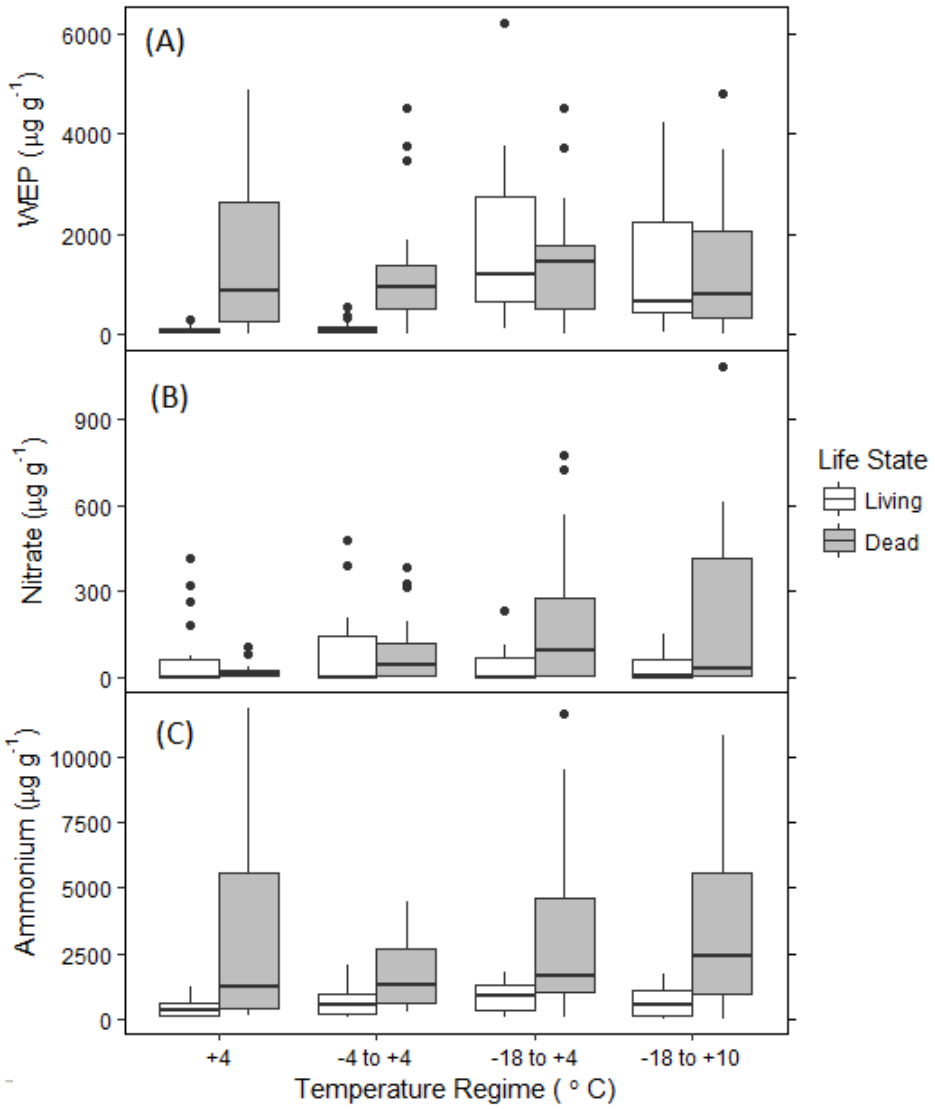

Figure 3.2 Nutrient [(A) water-extractable P (WEP), (B) nitrate-N, and (C) ammonium-N] content extracted from cover crop tissues after exposure to various freeze-thaw cycle magnitudes, split by life state (living, terminated). All species are grouped. Boxplots show the 25th, 50th and 75th percentiles, whiskers show the 10th and 90th percentiles, and circles show outliers.

Termination significantly increased the release of NH_4-N compared to non-terminated samples ($F = 49.46$, $p \leq 0.001$; Figure 3.2, Table 3.1c). When both living and terminated samples were pooled (in the 2-way ANOVA), significant differences in extracted NH_4-N were found with the FTC treatment (Table 3.1c; $p > 0.05$); however, post-hoc analyses revealed that this was driven by a difference between the control (immediately extracted) samples and other FTC treatments and no differences were observed

between the other three FTC treatments. Extractable NO_3-N content was not significantly affected by termination, although heavy frost resulted in more variability in terminated samples than living samples (Figure 3.2). Of the two forms, NH_4-N contributed a larger portion of the N release, with over an order of magnitude of difference between NH_4-N and NO_3-N.

3.4.3 Differences in Extractable Phosphorus and Nitrogen Concentrations among Species and Life State

Nutrient release depended on CC species, their life state and magnitude of FTC (Table 3.1). Figures 3.3-3.5 only compare data from two FTC treatments as no significant differences were observed between the 4 °C and -4 to 4 °C treatments, and the -18 to 4 °C and -18 to 10°C treatments. When living (non-terminated) CC were exposed to minimal frosts no significant differences (Table 3.1e) were observed in WEP concentrations across species (Figure 3); however, when plants were exposed to heavy frosts *(e.g.* exposure at -18 to 4 °C), stratification occurred with significant differences existing among species ($F = 13.98$, $p \leq 0.001$, Table 3.1e, Figure 3.3b). While all living species shared the same trend of increased WEP availability under extreme freezing conditions, the range in magnitude varied greatly. In living CC, from smallest to greatest release of P, the order of species was hairy vetch, red clover, cereal rye, oat, and oilseed radish (both shoot and root tissue had substantial WEP availability; Figure 3.3b).

Species differences were also apparent in terminated samples (Table 3.1e). Under both temperature treatments, terminated samples of red clover, cereal rye, oat, and oilseed radish shoot were all affected in a manner that was similar to living samples exposed to heavy frosts (Figure 3.3) indicating that heavy frost and termination achieve the same effect in these species. However, hairy vetch and oilseed radish root differed in WEP release when terminated compared to living tissue (Figure 3.3c,d). WEP release from terminated oilseed radish root remained quite small under minimal frost, unlike its shoot tissue counterpart, irrespective of termination. The effects of heavy frost on living and terminated oilseed radish root were comparable. Alternatively, terminated hairy vetch samples had significantly greater WEP availability than living samples, even under light frosts ($F = 55.63$, $p = 0.002$).

Indeed, the effect of heavy frosts did not result in further increases compared to light frosts as there was no significant difference between sprayed hairy vetch frozen at to -4 °C and samples frozen at -18 °C.

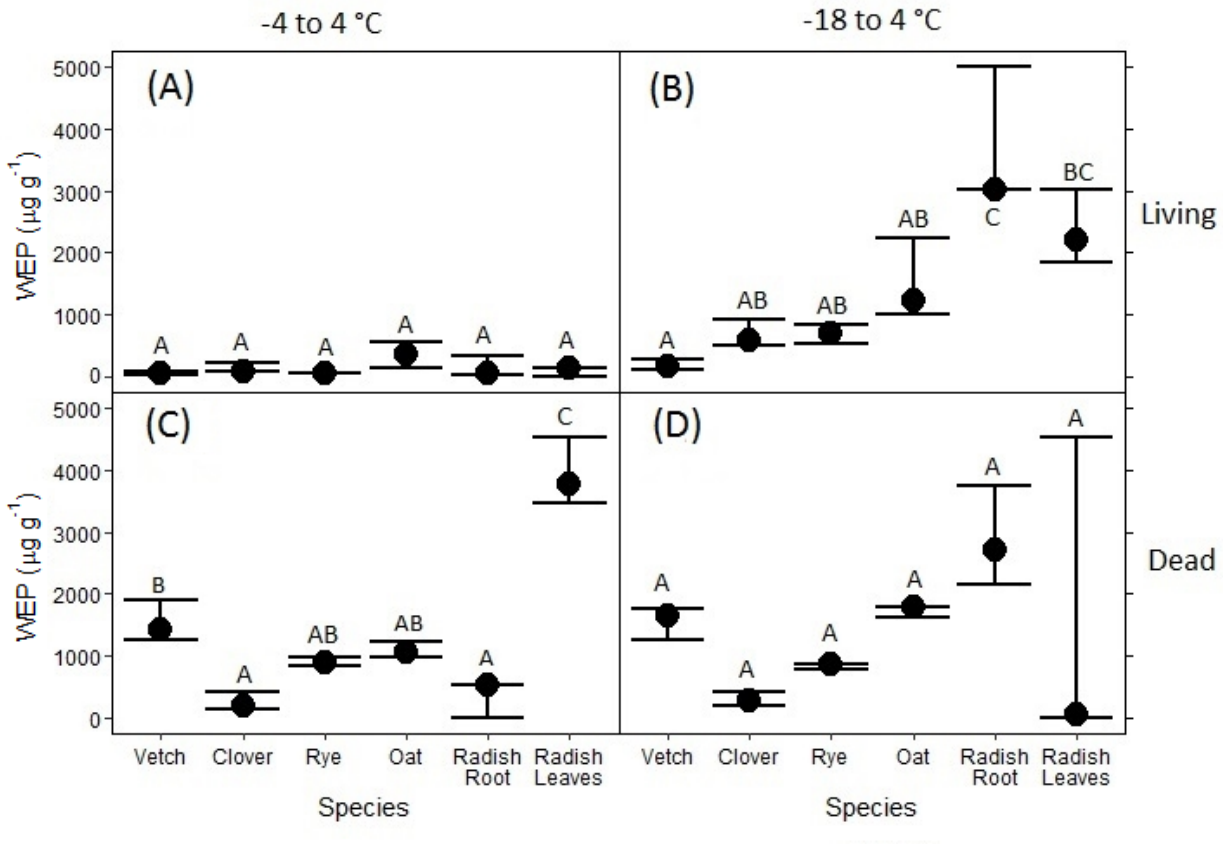

Figure 3.3 Median (symbol) and 95% confidence interval (error bars) of water extractable phosphorus (WEP) concentrations extracted from different cover crop species. Panels indicate different magnitudes of freeze-thaw cycle exposure and life-state: (a) living samples exposed at - 4 to 4°C, (b) living samples exposed at -18 to 4 °C, (c) dead samples exposed at -4 to 4 °C, (d) dead samples exposed at -18 to 4 °C. In each panel, shared letters indicate no significant differences between species determined through the Bonferroni Correction.

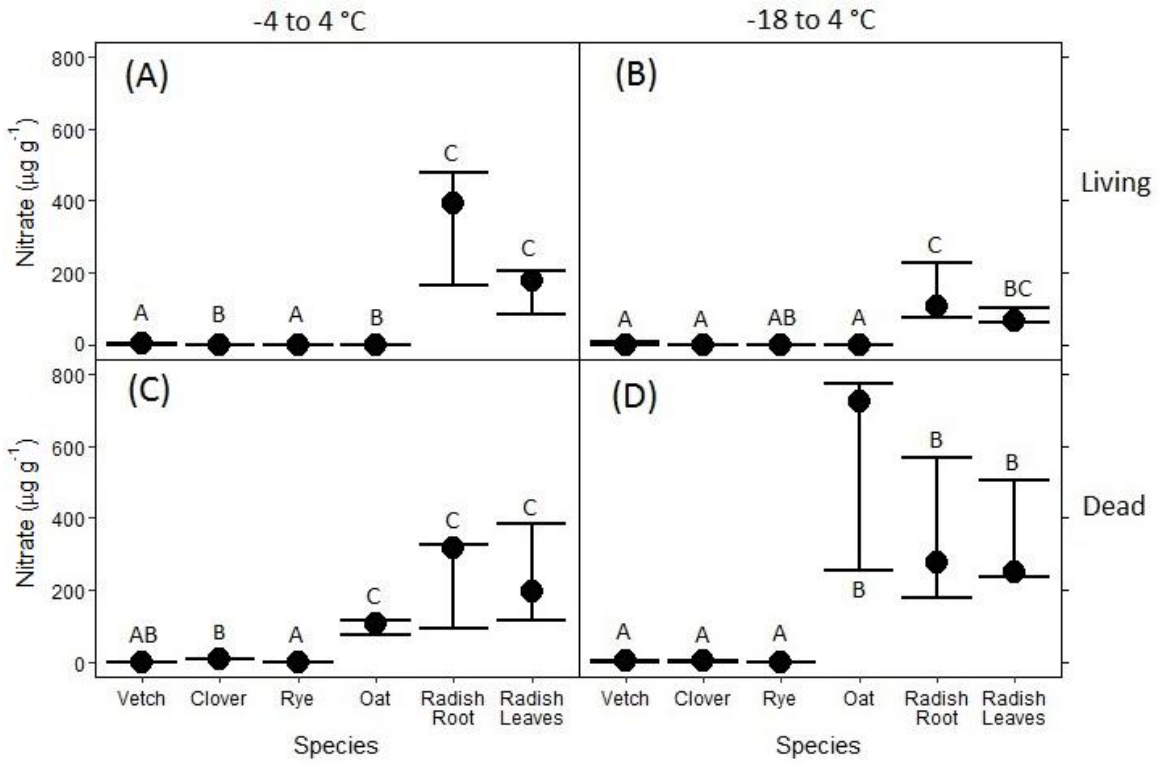

Figure 3.4 Median (symbol) and 95% confidence interval (error bars) of nitrate-N concentrations extracted from different cover crop species. Panels indicate different magnitudes of freeze-thaw cycle exposure and life-state: (a) living samples exposed at -4 to 4°C, (b) living samples exposed at -18 to 4 °C, (c) dead samples exposed at -4 to 4 °C, (d) dead samples exposed at -18 to 4 °C. In each panel, shared letters indicate no significant differences between species determined through the Bonferroni Correction.

Species differences in potential NO_3-N loss were clearly divided, with oilseed radish releasing relatively larger quantities from both living and terminated samples (Figure 3.4) relative to the other species. The sole exception was oat, which also released significantly more NO_3-N when terminated ($F = 25.96$, $p = 0.007$) under both FTC regimes. No general trends were evident between the two FTC intensities comparing species differences in NH_4-N loss in living tissue, except that hairy vetch had the smallest quantity of NH_4-N release under both FTC treatments (Figure 3.5a,b). Cereal rye (living samples) had a greater rate of NH_4-N release under light frost than all species except oat, but under heavy frost, oat and oilseed radish (shoot and root) increased to similar concentrations (Figure 3.5a,b). However,

when terminated, oilseed radish and oat both act as greater sources of NH_4 than other CC and similar to how termination affected WEP availability (Figure 3.5c,d).

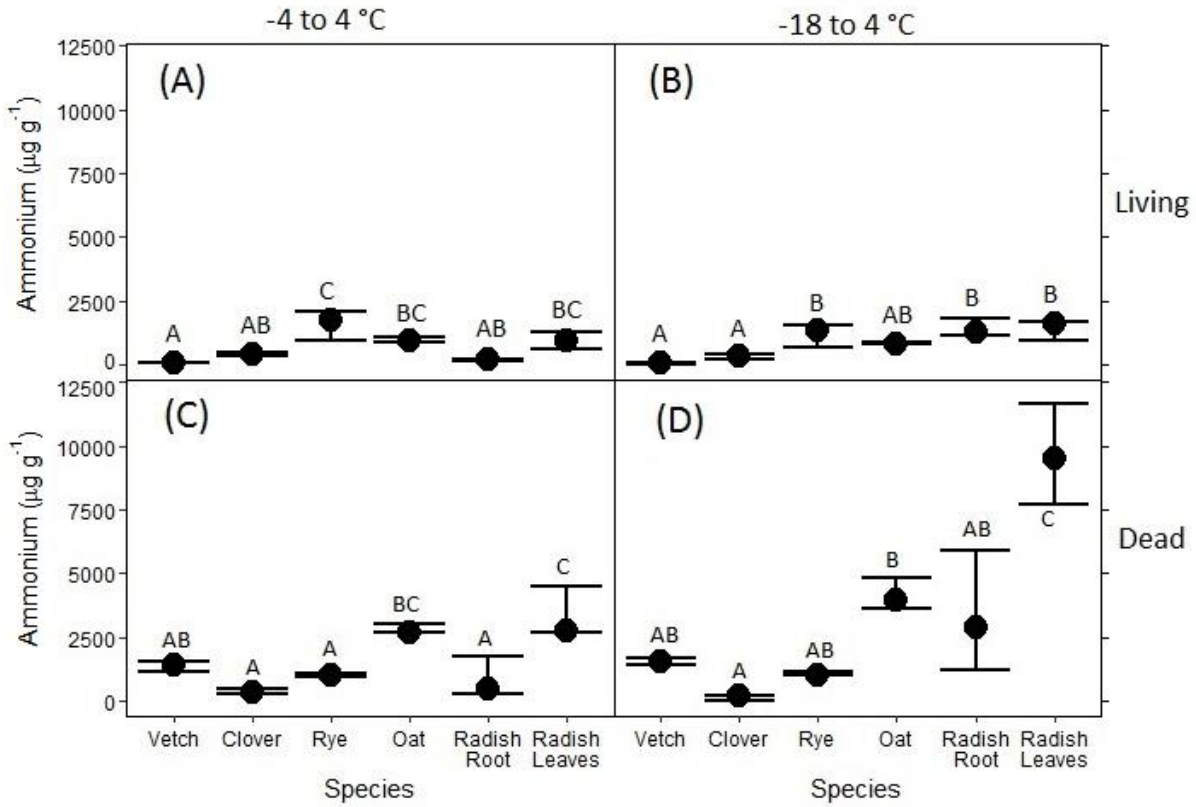

Figure 3.5 Median (symbol) and 95% confidence interval (error bars) of ammonium-N concentrations extracted from different cover species. Panels indicate different magnitudes of freeze-thaw cycle exposure and life-state: (a) living samples exposed at -4 to 4°C, (b) living samples exposed at -18 to 4 °C, (c) dead samples exposed at -4 to 4 °C, (d) dead samples exposed at -18 to 4 °C. In each panel, shared letters indicate no significant differences between species determined through the Bonferroni Correction.

3.4.4 Field Biomass and Total Phosphorus Pools

When nutrient concentrations were combined with estimated biomass data (Table 3.2), there was substantial variability in the magnitude of TP available among different species; however, this was the result of larger differences in biomass than the observed variability in TP content. Field scale WEP pools were estimated at to vary from 0.1 to 1.75 kg ha^{-1} with light frost, and up to 13.1 kg ha^{-1} under heavy frost conditions. After exposure to minimal freezing temperatures, ≤ 10% of TP was available as

WEP across all CC species. In contrast, heavy frost greatly increased the range in potential WEP availability (Table 3.2). While almost no change occurred in the hairy vetch (≤ 3.9%), 21-38% of TP was found as WEP in the red clover, cereal rye and oat, and as much as 51% (shoot) and 100% (root) of the TP was extracted from oilseed radish tissue.

Table 3.2 Potential field-scale water extractable P (WEP) and total P (TP) pools from measurements of cover crop dry biomass from plot-scale field studies. Median WEP concentrations from three replicates were taken from each CC species for three of the freeze-thaw cycle intensities. Biomass included all shoot tissue aboveground and oilseed radish root biomass only included the portion of the root which protruded from the soil. All values are presented in kg ha^{-1}

Species	Dry biomass	TP Pool	WEP at 4 °C (% of TP released)	WEP at -4 to 4 °C (% of TP released)	WEP at -18 to 4 °C (% of TP released)
			kg ha^{-1}		
Hairy vetch	1700	9.4	0.09 (0.95%)	0.10 (1.1%)	0.37 (3.9%)
Red clover	1700†	4.6	0.07 (1.5%)	0.17 (3.7%)	1.2 (27%)
Cereal rye	1900	7.6	0.26 (3.5%)	0.13 (1.7%)	1.6 (21%)
Oat	4600	19	0.85 (4.5%)	1.75 (9.2%)	7.1 (38%)
Oilseed radish shoot	4800	26	0.46 (1.8%)	0.66 (2.5%)	13.1 (51%)
Oilseed radish root	1200	4.6	0.10 (2.1%)	0.07 (1.6%)	4.6 (100%)

† Red clover biomass was estimated

3.5 Discussion

3.5.1 Importance of the Magnitude of Temperature on Extractable Phosphorus and Nitrogen Availability

The results of this study are in agreement with previous studies that have reported positive relationships between FTC magnitude and WEP content. In the current study, the low-magnitude FTC (-4 ^{o}C) does not appear to have been sufficient enough to cause substantial damage to plant cells, whereas the more extreme FTC temperatures (-18 ^{o}C) readily damaged plant cells, leading to elevated losses of P (Tukey and Morgan, 1963; Bechmann et al. 2005; Liu et al., 2013a; Riddle and Bergstrom, 2013; Liu et

al., 2014; Lozier et al., 2017). The temperature at which plants were thawed appeared to be less important than freezing temperature as no differences were observed between samples thawed at cooler or warmer temperatures. Indeed, in the current study, plant samples were fully thawed within 24 hr at 4 ^{o}C, irrespective of the freezing temperature (-4 °C and -18 °C).

Liu et al. (2014) indicate that the use of -18 °C is an appropriate freezing event for evaluating CC usage in Nordic regions; however, it is less appropriate for simulating the climate conditions of more temperate regions such as the Great Lakes region of North America. Indeed, although temperatures of -18 °C occur in the Great Lakes region, they are uncommon. Long-term (30-year mean, 1981-2010) data for Waterloo, ON (43.45 N 80.38 W) indicate that daily low temperatures are typically on the order of -10 °C or greater (Environment Canada, 2015a). Although colder temperatures (e.g. -25 °C) occur, CC in the Great Lakes region of North America are typically protected from extreme weather conditions by an insulating layer of snowpack. In Waterloo, ON, snow depths of ≥ 10 cm have been observed for over a third of January and February, and depths ≥ 5 cm are common from the end of December to early March (Environment Canada, 2015a). Thus, the potential of CC exposure to FTC in the Great Lakes region primarily occurs prior to snow cover in December, and during the spring melt of March, during which temperatures are at or near freezing overnight, and slightly above freezing during daytime. As the results of this experiment indicated that minimal freezing (-4 °C) did not have substantial effects on WEP levels, it reasonable to suggest that potential for CC to act as a P source in winter may be highly dependent on regional climate and indicates that regional CC-specific nutrient BMPs are needed. Indeed, field studies in Canada that have demonstrated substantial P loss from CC in winter have been conducted in regions with severe winter conditions (northern Great Plains, *e.g.* Tiessen et al., 2010; Liu et al., 2013b) where vegetation are killed by frost and subsequently exposed to harsh frost conditions until the thin snowpack thaws. In fields located in the Great Lakes region where winters are milder, Lozier et al. (2017) observed that even in a very cold winter, temperatures beneath the snowpack were

moderate. In contrast, the negligible impact of FTC magnitude on extractable N concentrations suggests that regional differences in climate should not be an important factor in choosing to use a CC or which species to use for N management in terms of N losses from plant tissues.

It is important to note that the extreme conditions used in the current study (and others like it) represent the near-maximum amount of nutrients that can be released from the plants, and the potential for WEP release may be less under field conditions. Indeed, the additional stress on plant tissues from clipping roots and the laboratory shaking extraction procedure likely elevated the observed WEP availability compared to what may be observed under natural conditions. Moreover, other processes may occur that may impact edge-of-field losses originating from CC. For example, in fields, decaying vegetation are connected to root systems, plants may desiccate in fields prior to surface runoff, and P released by plants may be retained by the soil.

3.5.2 Impact of Herbicide Application on Extractable Phosphorus and Nitrogen Availability

Comparing the effects of termination and FTC magnitude (Figure 3.2), it was apparent that termination can result in greater levels of P release than living plants when only slight losses would otherwise occur from light frost. This suggests that termination should be avoided in regions where heavy frost is unlikely to occur, as it removes the ability of a CC to resist the impact of freezing temperatures. In regions with harsh climates, the termination of CC prior to winter may be a BMP for mitigating P release from CC by enabling the plants to release the P pool prior to the onset of the heavy winter frost. In Ontario, Canada and the Northern Great Plains, surface runoff is typically associated with winter thaws and the main snowmelt event (Liu et al., 2014; Van Esbroeck et al., 2017), increasing the potential for P release from plants due to increased contact time between vegetation and runoff water (Liu et al., 2014; Lozier and Macrae, 2017). If the vegetation P pool is released prior to this occurrence, the leached P is more likely to infiltrate and be adsorbed in the soil and thus retained in the field.

In nearly all species tested, with the exception of hairy vetch, termination resulted in WEP release that was in the same range as concentrations extracted after being exposed to -18 °C FTC. Therefore, herbicide induced senescence does not normally make additional P available for extraction, and appears to only make available P that would also be leached by other cellular disruption. Hairy vetch was observed to be the sole exception to this, as greater concentrations were leached after termination than in living tissues (Figure 3.3c,d). In fact, after termination, hairy vetch became an equivalent P source to oat and oilseed radish. As red clover and cereal rye did not release additional P when sprayed with herbicide in the current study, they may be more suitable winter CC for farmers who require that plants are killed in autumn. However, switching from a fall herbicide application to spring termination may be more effective at retaining P with hardier, frost-tolerant CC species in moderate climates.

In the case of N, the potential for terminated CC to release elevated levels of NH_4-N was evident in comparison to living crops (Figure 3.2). Indeed, termination appears to be a more important factor to consider than exposure to heavy FTC (Figures 3.4, 3.5). Thus, avoiding fall termination of a CC should be considered a BMP for minimizing N loss.

For both N and P, other management practices such as mowing or tillage may be used to terminate CC (Blanco-Canqui et al., 2015), which should be compared to herbicide application in future work. Future experiments should evaluate the practice and method of termination at a larger scale using in field studies to determine the true extent of leaching in dead CC.

3.5.3 Influence of Species on Extractable Phosphorus and Nitrogen Release

Variation between replicates in controls (plant samples extracted immediately, not exposed to FTC) indicated that some degree of natural variation exists. It seems possible that some part of the intra-species variation occurred as a result of using both stem and leaf tissues, because stems and leaves may have different P and N contents (Roberson et al., 2007; Øgaard 2015). While best effort was made to

maintain a consistent ratio of stem and leaf tissues, this may remain a source of variation. Despite this, it remains important to consider both stems and leaves, as stems can make up a substantial portion of aboveground biomass, and the greater lignin content of some species may provide important resistance to FTC effects on P leaching (Liu et al., 2014) and presumably N release.

Variation in P content was observed between species, which suggests that some species may pose a greater risk for P export, and others may serve as suitable candidates for mitigating P export. The relative ranking of hairy vetch< red clover <cereal rye < oat <oilseed radish was similar to Miller et al. (1994) who also found less WEP in red clover than oilseed radish. As expected, a distinct difference emerged with species that do not winter-kill releasing less P than the two CC, oat and oilseed radish, which are readily killed by cold temperatures. However, in addition to CC species, other factors such as stage of development and acclimation to cooler temperatures influence susceptibility to freezing temperatures. As neither oat nor oilseed radish had large increases in P release between control (immediately extracted) samples and minimal freezing, the experimental temperature of -4 °C may not have been cold enough to damage plant tissues to the point of significant P release. Despite this, quantities of P leached from heavy freezing events (-18 °C) were substantially greater in oilseed radish than other species, and suggest that oilseed radish should be avoided in climates that have such extreme temperatures without insulating snow cover and where P export is of pressing concern. Additionally, the tap root of oilseed radish may pose greater risk than shoots, as 1) they are typically a large part of plant biomass; 2) the roots may release WEP at greater levels than oilseed radish shoot tissues; 3) the roots are partially emergent, and are therefore not fully insulated by soils as other CC roots would be. In situations where ambient temperatures drop to around -18 °C and there is no snow cover present, oilseed radish roots present tremendous risk for leaching of dissolved P to occur. Alternatively, oilseed radish can be managed by farmers to minimize tap root growth by increasing seeding density or choosing seed selections which do not produce large tap roots.

While oat does not have the same degree of risk of P loss as oilseed radish, it also appeared to be a weaker candidate species in terms of P release. Because P concentrations in leachate were on average approximately twice as large from the control (immediately extracted) to samples frozen at -4 °C, there is risk that minimal to moderate freezing in field conditions may result in dissolved P leaching. Indeed, other studies have found that oat releases some P under light frosts in both field (Lozier et al., 2017) and laboratory settings (Lozier and Macrae, 2017). However, oat CC naturally senescence when exposed to freezing temperatures; which may dry out shoot tissues and prevent FTC from damaging cells, possibly reducing the potential for WEP release as seen in other species (Miller et al., 1994; Elliot, 2013). Although red clover and hairy vetch greater P release when comparing the controls (immediately extracted) to minimally frozen samples, P loss was minimal and both species seem to be suitable candidates for further field testing.

3.5.4 Field Biomass and Total Phosphorus Pools

Cover crop growth and estimates of field biomass were consistent with other studies in the Great Lakes region (Vyn et al., 1999; Snapp et al., 2005; Belfry et al., 2017; Coombs et al., 2017). In addition to the greater availability of WEP in oat and oilseed radish in lab extractions, the relatively larger density on field indicates greater enhanced risk for P loss from fields during the winter season. The combination of relatively large TP content but smaller WEP availability in hairy vetch highlights it as a prime candidate species for reducing P loss over other red clover and cereal rye, the other two species tested that overwinter in temperate climates. The use of legumes as a CC should be done with caution. Indeed, Meisinger et al. (1991) suggested that the use of legumes could be problematic for N leaching due to their impacts on soil mineral N concentrations. However, Vyn et al. (1999) and Coombs et al. (2017) reported that legumes did not increase soil mineral N in autumn in temperate climates.

To date, most studies on winter P or N release from cover crops have been conducted in a laboratory setting and fewer field studies have been published. As noted earlier, laboratory

investigations such as the current study represent the initial stage of P loss from a field and may not directly translate to elevated edge-of-field losses. More information is needed on the relative contributions of vegetation and soil to field-scale P loss, and, if and how this varies with CC species, topography, soil test P and/or texture. A recent study (Lozier et al., 2017) found that in soils with relatively small soil test P, concentrations of P release from red clover and oat exceeded those from soil; however, when biomass or soil bulk density were considered, the soil represented a greater P source to surface runoff than CC. Lozier et al. (2017) also demonstrated that despite the release of WEP from CC and soil in winter, very little of the released P left the field, presumably due to interactions between infiltrated water and soil. Although the capacity for soils to significantly reduce the loss of P from CC has been demonstrated in laboratory settings (Bechmann et al., 2005; Riddle and Bergstrom, 2013), few field studies exist. The determination of the ability of CC to reduce P export in a variety of settings (soil types, climate zones) will require more field studies where losses of both dissolved and sediment-bound P species are compared between fields with CC and fields that are left bare over winter.

3.6 Conclusions

Cover crops have many benefits that must be balanced with the potential impact of nutrient losses to aquatic environments. This study has shown that cover crops may pose a risk for winter P release, but not N release; however, the potential for P loss varies with both species and frost magnitude. This suggests that in regions with mild or moderate winter climates, such as the Great Lakes Region, cover crops should remain an agricultural BMP, whereas in colder regions, cover crops should be used with caution. In this study, plants exposed to a heavy frost (-18 ^{o}C) had considerably greater concentrations of extractable P compared to those that were unfrozen (4 ^{o}C) or exposed to minimal frost (-4 ^{o}C). This was more apparent in frost-intolerant species such as oilseed radish, and less apparent in hardier species such as hairy vetch, red clover, and cereal rye. In contrast, termination by an herbicide released both P and N from all cover crops, irrespective of frost magnitude. This suggests that

termination should be avoided as a management practice in regions that only experience light frost, whereas fall termination may be more suitable for colder regions to mitigate snowmelt-related losses of P. Nutrient-specific BMPs (*i.e.* the use of cover crops to manage N) can result in unintended consequences such as P release in winter. This study has shown that both N and P management, as well as regional climate should be considered in the selection of both cover crop species and the use of autumn termination.

Chapter 4- Winter phosphorus release from different cover crop species relative to soil, runoff, and shallow groundwater phosphorus content in a temperate region

4.1 Overview

Cover crops (CC) have been shown to have agronomic benefits; however, the potential for increased loss of dissolved reactive P (DRP) has been shown following freeze-thaw cycles (FTC). This field study, conducted over one non-growing season (NGS) in Ontario, Canada, characterized water extractable P (WEP) concentrations in different CC species planted as monocultures, a biculture, and a polyculture, and compared observed changes in plant WEP pools to changes in P content in soil, surface runoff, shallow soil-water and shallow groundwater. Seven plots (0.4 ha) were established following winter wheat (*Triticum aestivum* L.) harvest. Throughout the NGS (Aug-Apr), CC shoot tissues and surface soil were routinely sampled for WEP analyses, and water samples (surface flow, infiltrate, and shallow groundwater) were collected following rain and snowmelt. Response to FTC varied among CC species, with increased [WEP] in frost-intolerant species but not frost-tolerant species. However, the top 5 cm of soil had a greater WEP pool than plants, and the variability in CC WEP pools over the NGS was not reflected in soil or water P concentrations, and, runoff P concentrations did not differ between monocultures and mixtures. Degree of FTC exposure should be considered in the selection of CC species in cold regions, however, in temperate regions, P release from vegetation does not appear to lead to increased P loss in runoff.

4.2 Introduction

In freshwater environments, degraded water quality and the eutrophication of inland lakes has been linked to elevated P loads from surrounding watersheds (Smith et al., 1998; Bennett et al., 2001; Anderson et al., 2002; Baker et al., 2014). Despite ongoing management efforts, record levels of algal growth has occurred in Lake Erie, in North America, as recently as 2011 and 2015, and is expected to worsen with climate change (Michalak et al., 2013; Smith et al., 2015). In 2014, a bloom near the intake of the water treatment plant for the City of Toledo, Ohio, USA shut off the water supply for over 400,000

people that relied on Lake Erie for drinking water (Jetoo et al., 2015). Many tributaries within the Lake Erie watershed consistently have P concentrations that exceed provincial guidelines (Baker et al., 2014; Chomicki et al., 2016; Maccoux et al., 2016; Chilima et al., 2017). Phosphorus loading into tributaries comes from a variety of sources, but is dominated by inputs from agricultural land use (International Joint Commission, 2014). To combat P loss from agricultural fields, numerous best management practices (BMPs) have been recommended. The use of cover crops (CC) throughout the non-growing season (NGS) is recommended to improve soil health and nutrient status, but also to minimize soil erosion and the associated transport of P in agricultural runoff (Sharpley and Smith, 1991; Blanco-Canqui et al., 2015). However, there is concern regarding the efficacy of CC in the reduction of P loss, as they have been shown to release dissolved P during the winter months, potentially leading to field P losses (Tiessen et al, 2010; Elliot, 2013; Liu et al., 2014).

Cover crops have the potential to reduce field P loss in two ways: through the reduction of soil losses from both wind and water erosion (Gyssels and Poesen, 2003; De Baets et al., 2011), and the uptake of dissolved species into plant biomass (Villamil et al., 2006). The retention of surface soil keeps particulate P (the largest P fraction) on fields where it may be taken up by the subsequent crop or be bound to the soil (Sharpley and Smith, 1991). In the case of dissolved P species, labile P is scavenged and held in the growing CC throughout the NGS, until the plant decays and nutrients are released into the soil P pool (Blanco-Canqui et al., 2015), ideally supporting crop growth the following year.

However, evidence suggests that when CC are grown in cold regions, freeze-thaw cycles (FTC) can increase the availability of P, which can be mobilized by runoff (Miller et al., 1994; Bechmann et al., 2005; Roberson et al., 2007; Elliot, 2013; Riddle and Bergstrom, 2013; Øgaard, 2015; Lozier and Macrae; Lozier et al., 2017; Cober et al., 2018). As ice crystals repeatedly form, plant cells may be lysed and water moving over these damaged tissues may leach nutrients; however, other nutrients, such as nitrogen

may be affected less than P in FTC conditions (Tukey and Morgan, 1963; Miller et al., 1994; Cober et al., 2018). Multiple factors may influence the potential release of P from a CC, including: number of cycles (Bechmann et al., 2005), the intensity of freezing (Øgaard, 2015; Cober et al., 2018), the species of CC (Liu et al., 2014; Cober et al., 2018), and degree of contact with water (Lozier and Macrae, 2017).

Although CC have been identified as potential sources of dissolved reactive P (DRP), this has largely been shown in laboratory experiments rather than field studies. Many of the studies that have demonstrated elevated P losses resulting from FTC have used harsh FTC temperatures, such as: -18 °C to room temperature (Riddle and Bergstrom, 2013), -18 to 18 °C (Liu et al., 2013; Liu et al., 2014), -18 to 10 °C (Bechmann et al., 2005). These temperature regimes indicate the potential for P loss, but may not reflect the true conditions that CC experience. Indeed, Liu et al. (2014) reported that most natural FTC occurs closer to 0 °C, which may have a lessened impact on P release. In a recent laboratory study, Cober et al. (2018) found significantly greater P release from CC exposed to harsh frost (-18 °C) in comparison to lighter frost (-4 °C), and suggested that the risk of winter P loss from CC may be reduced in regions that do not experience harsh winter frosts or that have significant snowpack to insulate plants.

Some work has been done to compare laboratory FTC experiments with natural conditions. For example, Roberson et al. (2007) found significant increases in plant P loss, from alfalfa (*Medicagosativa* L.), in lab extractions and simulated rainfall experiments; however, they did not observe this in a field setting, where natural variability in field conditions was greater. Øgaard (2015) compared plants exposed to seven days of FTC at -10 to 5 °C to plants left directly exposed to winter temperatures (without snow cover), and observed greater P loss in the laboratory study. Studies have also shown differences in the timing of P release by different CC species. For example, Øgaard (2015) observed that the greatest increase in WEP availability occurred in the early winter for most CC, but occurred later in

the season for two perennial species, indicating the potential impact of repeated FTC. Sturite et al. (2007) reported that P concentrations in leachate did not increase appreciably until spring snowmelt, despite numerous autumn FTC events. In a recent field study, Lozier et al. (2017) observed two major increases in WEP concentrations from CC: a slight increase occurred after the first heavy (-10 °C) frost, and a second larger increase during snowmelt; however, the major runoff events were not coincident with the timing of greatest WEP pools in plants. These findings suggest that although laboratory studies are important and permit researchers to constrain specific conditions, such laboratory studies may not reflect what is observed in a field setting. An improved understanding of how CC may respond to natural NGS conditions, as plants senesce throughout the winter in a field setting is needed.

In addition to the need for field studies on P release from vegetation, little field data quantifying the impacts of vegetation P on P loads in runoff exists. Phosphorus concentrations in overland flow have been strongly linked to P concentrations in surface soils (Sharpley et al., 1977; Uhlen, 1978; Sharpley et al., 1986). However, field studies in the Northern Great Plains in North America demonstrated elevated DRP concentrations in surface runoff during snowmelt on perennially frozen soils where infiltration is impeded by concrete frost (Trimble et al., 1958; Tiessen et al., 2010) However, in the temperate Great Lakes Region, Lozier et al. (2017) found that despite the presence of a large P pool in CC on fields, edge-of-field P losses in tile discharge and surface overland flow were small, demonstrating the potential for the retention of vegetation P by soils. Roberson et al. (2007) found similar results in a field study with alfalfa CC, where runoff P concentrations did not increase following repeated FTC, which may have been the result of extremely high natural variability in runoff P concentrations, or the infiltration and storage of P within the soil as opposed to runoff. The potential for extracted P to be held within soils has been well demonstrated, particularly when leachate is able to infiltrate into the soil matrix, as opposed to moving overland (Sharpley, 1995). The sorption of P to iron and aluminum oxides, as well as precipitating in calcium compounds works to buffer nutrient movement through subsurface soils

(Edzwald et al., 1976). In laboratory experiments, P loss from CC has been shown to be significantly lower when leachate is passed through soil cores (Bechmann et al., 2005; Riddle and Bergstrom, 2013). Thus, P losses may be lowered if leaching from a frozen CC occurs before the soil is frozen and the storm event does not generate overland flow (Roberson et al., 2007; Lozier et al., 2017). Given the potential of subsoils to buffer P in leachate (Andersson et al., 2013), the effects of vegetation P release during winter thaws are expected to be most apparent in surface runoff; however, given the control of soil P, slope position and soil texture on P concentrations in overland flow, it is unclear if the contribution of vegetation P to P concentrations in surface runoff will be observable. The simultaneous examination of plant and soil WEP pools and P concentrations in surface and subsurface runoff throughout the NGS in a field setting is needed to evaluate the efficacy of CC as a winter BMP.

The examination of different CC species, grown as either monocultures or mixtures is also needed. While many CC users plant monocultures, planting a polyculture, or mixture, can provide additional benefits to soil health (Blanco-Canqui et al., 2015). The impact of FTC on polyculture CC in field conditions is not well understood. It is possible that insulation from a dense frost-tolerant crop may reduce the effects of FTC on tender species. Moreover, Chapin (1980) suggested that nutrients leached from FTC could be taken up by living roots; thus it is possible planting frost tolerant species alongside CC that winterkill may potentially mitigate some of the P loss, whether from early senescence or during spring snowmelt events. Thus, a comparison of vegetation-soil-runoff patterns between monocultures and polycultures is needed.

The objectives of this study were to: (1) determine how WEP and plant TP contents in the field change throughout the NGS and if this varies among CC species; (2) examine P concentrations in soil, soil water (leachate) and shallow groundwater throughout the NGS and determine if changes in water chemistry coincide with changes in the vegetation P pool; and (3) determine if the observed changes in

plant and soil P for specific CC species differs when they are planted as monocultures, biculture, or polyculture.

4.3 Materials and Methods

4.3.1 Study Site and Experimental Treatments

This study was conducted in a field located near Bloomingdale, ON (43°30'30"N 80°28'12"W). Climate in the region is classified as warm-summer, humid continental (Dfb, Köppen Classification), with the mean annual maxima in July (20 °C) and mean annual minima in January (-7 °C). The region receives ~780 mm of precipitation annually, including 160 cm as snowfall between November and March (Environment Canada 30 year average, 1981-2010; Environment Canada, 2018). The field was under a corn (*Zea mays* L.)-soy [*Glycine max* (L.) Merr.]-winter wheat rotation on light Burford Loam/London Loam soils (Present and Wicklund, 1971). The field has a slope of 4%, draining into the adjacent Grand River. Surface soils have 26.5 +/- 8.3 mg kg^{-1} Olsen-P in the top 15 cm. The field was no-till, although periodic shallow vertical tillage (2") was done when crop residues were heavy. The field was not tilled prior to the planting of the CC. Fertilizer was applied to the field at the time of planting of winter wheat with a 2x2 band at a rate of 136 kg ha^{-1} (10 kg N, 58 kg P_2O_5, 67 kg K_2O per ha) in the fall of 2016, and a broadcast application of 134 kg ha^{-1} N in the spring of 2016.

The ~5 ha field was split into seven plots, each approximately 0.4 ha in size (Figure 4.1). The CC plots were planted on August 9, 2016, following winter wheat harvest. Four plots were planted as CC monocultures of cereal rye (*Secale cereal* L.) seeded at 67 kg ha^{-1}, oilseed radish (*Raphanus sativus* L. var. *oleoferus* Metzg Stokes) at 8 kg ha^{-1}, oat (*Avena sativa* L.) at 45 kg ha^{-1}, and hairy vetch (*Vicia villosa* Roth) at 22 kg ha^{-1}. Two plots were planted as mixtures of CC species: a biculture of cereal rye and oilseed radish at 37 kg ha^{-1}, and an eleven-species polyculture of cereal rye, oilseed radish, oat, hairy vetch, phacelia (*Phacelia tanacetifolia* Benth.), pea (*Pisum sativum* L.), crimson clover (*Trifolium incarnatum* L.), faba bean (*Vicia faba* L.), sunflower (*Helianthus annuus* L.), sorghum [*Sorghum bicolour*

(L.) Moench], and flax (*Linum usitatissimum* L.), at 50 kg ha^{-1}. A control plot was not planted, but had some strips of volunteer wheat growth, which is typical of wheat production in North America.

4.3.2 Plant and Soil Sample Collection and Processing

Plant biomass was measured once during the field season, on October 12, 2016. Three 0.25 m^2 quadrats were clipped from each plot. Oilseed radish shoot and root tissue were taken from the same plot and weighed separately. Only root tissue which protruded above the soil surface was collected. Within the polyculture, CC species were categorized as non-legume broadleaves, legumes, grasses, and oilseed radish roots. Because no emergent roots were found in any of the three quadrats from the paired rye and radish plot, the biomass for radish root from the 11-species mixture plot was substituted for calculating the plant TP and WEP pools and is included in the value listed in Table 4.2. These biomass values were used to determine the CC P pools in units of kg ha^{-1}.

Plant and soil samples were collected at two-week intervals between September and December, and approximately monthly (Dec. – Apr.) once the snow pack had developed. No soil samples were collected during the January sampling campaign due to frozen ground that prevented sample collection. Plant samples were collected by clipping the whole shoot tissue at the surface, with the exception of oilseed radish which was separated by shoot and root tissue. For each sampling event, 10-20 individual plants were clipped throughout the plot and batched together to homogenize samples before extraction. Plant samples were extracted within 12 hr of collection. Shoot tissues were clipped into approximately 4 cm long pieces to fit into sample containers. Oilseed radish root samples were treated differently because of their substantial size, where cylindrical pieces of root, approximately 1 cm length, were cut and extracted individually. Samples from the biculture and polyculture were analysed in subsets. Living volunteer wheat from the control plot was collected and extracted with the other CC plant samples; no wheat residue from the previous crop was collected. At least 5 g of field moist material was shaken in 50 mL deionized water for 1 hr at 100 rpm, after which leachate was

immediately gravity filtered through Whatman No. 42 filter papers. Filtered samples were immediately frozen. A small subset of tissue from each CC species and sampling event was dried at 100 °C for 24 hr to determine water and TP content.

Three soil samples (0-5 cm), each consisting of three homogenized soil cores, were collected at random locations across each plot on each sampling event for the determination of WEP pools. Soil samples were oven dried at 30 °C before extraction. 10 g of dried soil was extracted in 50 mL of deionized water, at 100 rpm for one hour, and immediately filtered through Whatman No. 42 filters. Additional soil samples were taken in October 2017 for the determination of bulk density using standard techniques (12 cm diameter cores to 5 cm depth). In April 2017, six soil samples were collected, each consisting of fifteen homogenized soil cores taken from 0-15 cm depth and Olsen-P content was analysed using sodium bicarbonate extraction and ammonium molybdate colorimetric methods (A & L Canada Laboratories Inc.).

4.3.3 Environmental Data and Water Sample Collection and Processing

Soil probes were deployed September 20, 2016 on the field from three loggers (Figure 4.1) to monitor soil temperature and moisture content, as well as ambient temperature at 1 m above the surface (Decagon Devices Inc. 5TMSM/Temp sensors with EM-50 data logger, Onset Ltd.). Soil probes were installed in each of the seven plots at 5 cm, with an additional probe installed under the polyculture CC plot at 1 cm and 5 cm depth. Estimates of precipitation and snow cover were taken from a nearby monitoring station (9.5 km from the site, Climate Identifier 6149389, Environment Canada, 2017).

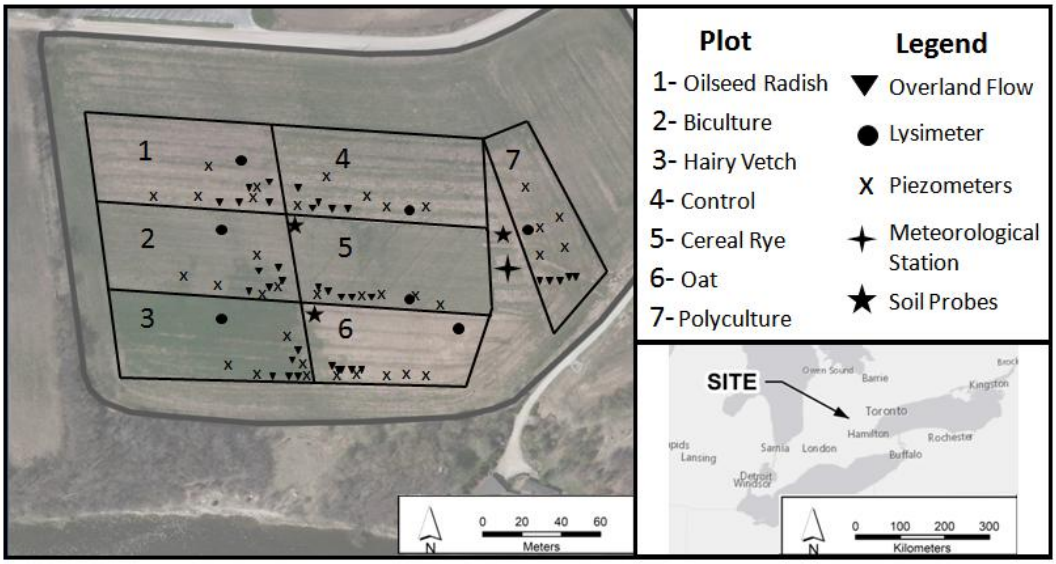

Figure 4.1 Map of the field site located at 43°30'30"N 80°28'12"W, in Bloomingdale, ON. The field was split into seven plots, approximately 0.4 ha in size, of different cover crop species, a biculture, and a polyculture. The position of water collection and climate monitoring equipment for each plot are indicated.

A variety of water sample collection units were installed between August and September 2016 to capture overland flow, shallow soil water/leachate (0-15 cm depth), and shallow groundwater flow (5-25 cm depth). Overland flow was collected using weirs that directed flow into 1 L nalgene bottles that were buried so that their lids rested ~0.5 cm below the surface to facilitate collection. Each bottle was equipped with a ping pong ball that sealed the 3 cm hole in the bottle lid when the bottle was full. Five overland flow collectors were installed in each CC plot. Because the field was sloped, and surface runoff typically does not occur at the plot scale on sloping ground, no efforts were taken to quantify surface runoff volume.

Infiltrated shallow soil water (leachate) in the top 15 cm of soil was collected in a single lysimeter installed in each plot. This was done to capture preferential flow that may not have been observed in samples collected from piezometers, which would be more reflective of matrix flow. In each lysimeter, an intact block of soil, approximately 30(L) x 22(W) x 15(H) cm, was placed onto nylon screening in a perforated polyethylene container that was nested in a second non-perforated reservoir

container that allowed leachate to collect in a 5 cm deep space below the soil block. Lysimeters were buried flush with the ground to minimize container effects. Early samples from the cereal rye plot lysimeter were discarded because mice were repeatedly found nesting in the container. The lysimeter was discarded and a new one was deployed.

Shallow ground water was sampled at five piezometer nests (screen depths 5-15 cm, 15-25 cm) in each CC plot. Piezometers were constructed from ABS pipe (3.8 cm ID) triple wrapped with screening. Each piezometer had a non-perforated 200 mL reservoir below the screening depth to collect samples during periods when the water table rose and fell rapidly before a field visit could be done. Piezometers were developed 2-3 times before samples were collected using a peristaltic pump. In the control plot, all piezometers and the lysimeter were installed in bare areas where no volunteer wheat was present.

All water samples were collected within 24 hr of a storm event ending and after sample collection each collector was purged. A 50 mL subsample of each sample was filtered through a 0.45 µm cellulose acetate filter within 24 hr of collection. Filtered samples were immediately frozen prior to analysis for DRP. In the surface runoff samples, a second 50 mL subsample was left unfiltered and acidified to 0.2% sulfuric acid prior to digestion for TP analysis.

4.3.4 Laboratory Analysis

For the determination of plant total P pools, plant digestions were done using sulfuric acid/hydrogen peroxide/lithium sulfate/selenium methods (Parkinson and Allen, 1975). For the determination of TP in overland flow, water samples were digested using acid persulfate digestions and an autoclave (EPA/600/R-93/100, Method 365.1). Phosphorus concentrations from plant and soil extractions, plant digestions, and filtered water samples were analysed using ammonium molybdate/ascorbic acid colorimetric methods Bran- Luebbe AutoAnalyzer III system, Seal Analytical, Methods G-103-93 (DRP), G-188-097 (TP)].

4.3.5 Statistical Analysis

A series of analysis of variance (ANOVA) tests were run to determine if plant or soil WEP pools varied with CC species or time. The specific tests run are noted in the results section when the test results are reported. In the data, normality was evaluated with a one sample Kolmogorov-Smirnov test. Where data were not normally distributed, data were log-transformed and re-tested. When normality could not be assumed, data were ranked and non-parametric tests were used instead. When data were normal, two-way parametric ANOVAs were run, with the Bonferroni correction applied post-hoc. If a significant interaction existed between the two factors, data were split and one-way ANOVAs were run on each level of the factors. When data were non-normal, the non-parametric Scheirer-Ray-Hare test was used. Median DRP concentrations from water samples were correlated against median plant and soil WEP from samples taken prior to each water sampling event to evaluate the potential relationship between each of the potential P pools and runoff P concentrations with the Pearson product moment correlation. All statistical tests were done with an alpha value of 0.05 chosen to determine significance.

4.4 Results

4.4.1 Weather Conditions over the Study Period

Weather conditions experienced over the study period permitted the division of the field season into distinct periods with varying degrees of FTC intensity (Early, Mid, Late, Figure 4.2). The “early” period (Oct. – Dec.) was characterized by dry conditions preceding October, and generally small precipitation events (with the exception of a single large rain event in November). Although temperatures fell below freezing during this period, temperatures remained generally close to zero with the exception of two nights during which temperatures fell to between -5 and -10 °C in October and November. The “mid” period commenced with the accumulation of a snowpack (mid Dec.). Throughout this period, air temperatures dropped as low as -20 °C, but surface temperatures stayed above freezing until a January thaw melted the insulating snowpack. Freeze-thaw cycles, ranging mostly from -15 °C to 5 °C, occurred throughout the mid period, and overland flow and subsurface drainage were generated

on several occasions between January and March. The “late” period occurred at the end of the NGS (Mar. – Apr.) and was dominated by warm temperatures with low intensity frosts (-2 to 10 °C), with one period of colder frost (-10 °C). Despite over 17 cm of snow falling in March, warmer temperatures prevented the accumulation of snowpack, exposing CC to high levels of rainfall, snow-melt, and warm daily temperatures. Several runoff events occurred during the late period. Approximately 600 mm of precipitation (160 mm as snow water equivalent) fell during the entirety of the study period, compared to the average from 1981 to 2010 of 500 mm of precipitation with 160 mm of snow water equivalent (Environment Canada, 2018).

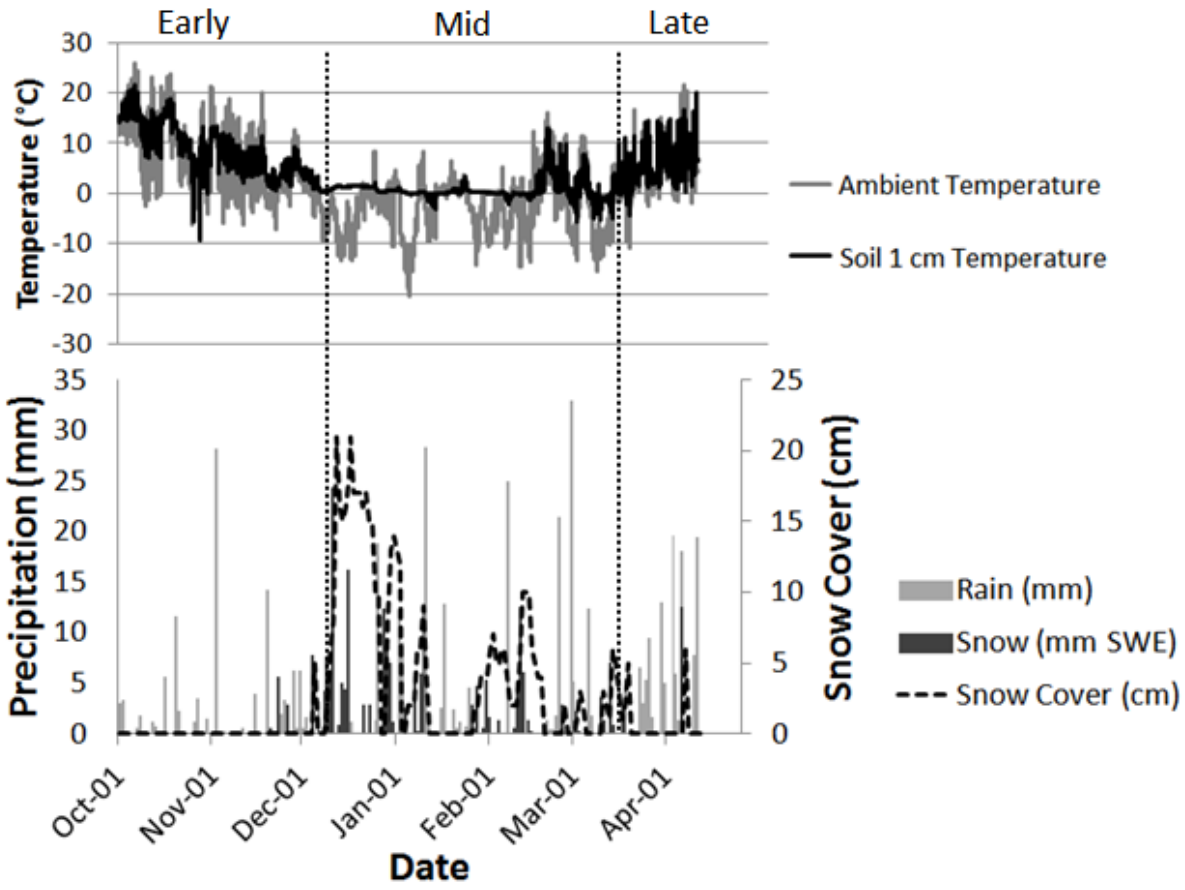

Figure 4.2 Weather data from the field site showing ambient and soil (1 cm depth) temperatures (top), and precipitation [rain and snow water equivalent (SWE)] and snow cover (bottom) throughout the 2016/17 non-growing season. Dotted lines indicate the periods of the non-growing season.

4.4.2 Phosphorus Release from Different Cover Crop Species

Before exposure to FTC (the first sampling event), there was little difference in WEP availability among the different CC species, and median WEP pools for all CC species were less than 0.25 kg ha^{-1} (Figure 4.3). Following exposure to varying intensities of FTC, differences in peak WEP pools were observed among CC species with regard to the magnitude and seasonal timing of P release. When evaluated with a two-way ANOVA, the effects of seasonal period and CC species resulted in a significant interaction (Table 4.1a); therefore, separate one-way ANOVAs were run to determine if there were significant differences existed between CC species at different points of the NGS (Table 4.1b), and if each CC significantly differed in P availability during different periods of the NGS (Table 4.1c).

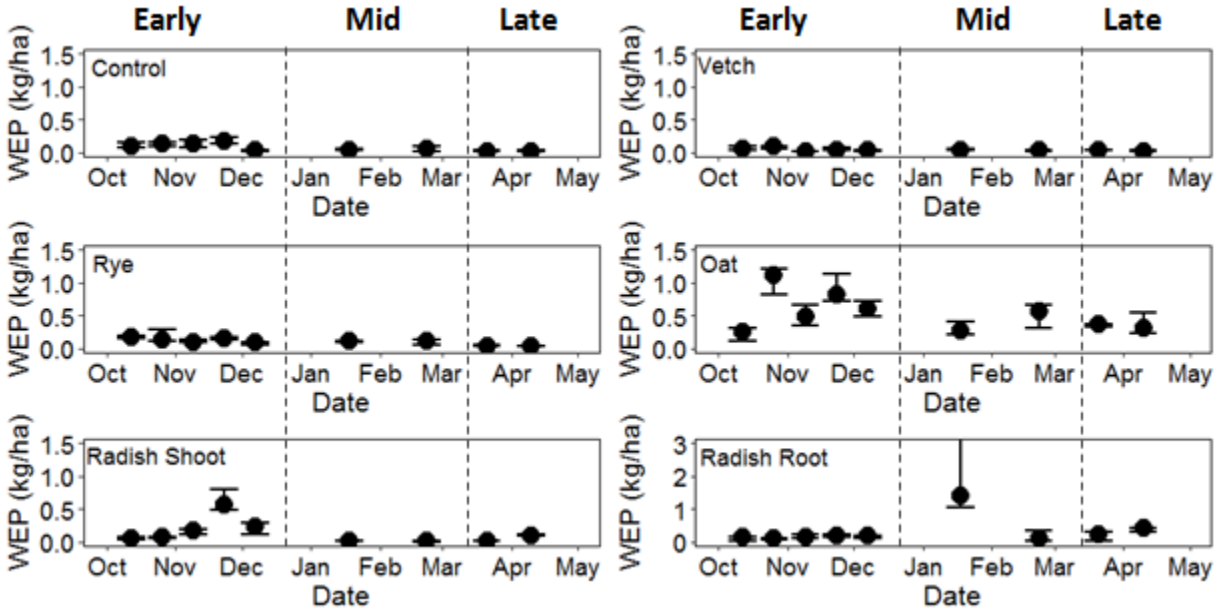

Figure 4.3 Median (point) and 95% confidence intervals (bars) for water extractable phosphorus (WEP) pools from cover crop samples of different species collected from a field site throughout the 2016/17 non-growing season. Sampling events have been split by periods of the non-growing season by weather conditions. Note the different scaling used for oilseed radish root.

Water extractable P concentrations differed with CC species in all three periods of the study (Table 4.1b). Of the CC species studied, the greatest WEP pools were observed in the root tissue of the oilseed radish (Figure 4.3); with slightly smaller WEP pools in the radish shoots and oat. Concentrations

of WEP expressed as μg g^{-} are shown in Appendix A; the greatest concentrations occurred in oat and radish root, whereas the larger WEP pool in radish shoot tissues was driven more by biomass than available P. In contrast, WEP pools from the volunteer wheat, vetch, and rye were not elevated. These differences were driven by a combination of differing WEP concentrations among species, and the large differences in plant biomass of over 1000 kg ha^{-1} (dry), between the CC plots (Table 4.2). For example, from the Feb 22 sampling, CC median WEP concentrations were 14 mg kg^{-1}, 58 mg kg^{-1}, and 124 mg kg^{-1}, from hairy vetch, cereal rye, and oat. The greatest WEP pool, seen in the oilseed radish root, was primarily driven by the 1150 mg/kg WEP released during the Jan 18 extraction.

Table 4.1 Results of ANOVA tests on the effects of non-growing season period, cover crops, and plot soil, and biculture mixing on water extractable phosphorus (WEP) release. Bolded results were found significant at an alpha value of 0.05. Where significant interactions were found significant in a two-way ANOVA the main effects were not considered. An * indicates the test was performed on log transformed data.

(A) 2-way ANOVA of period x CC species (monocultures) effect on release of water extractable phosphorus from plants:	*SS*	*Test Stat*	*Sig.*
Period	0.40	F= 2.48	.087
CC Species	5.25	F= 13.15	**.000**
Period x CC Species	4.24	F= 5.31	**.000**
(B) 1-way ANOVA of CC species effect on release of water extractable phosphorus from plants:	*SS*	*Test Stat*	*Sig.*
Early*	12.01	F= 24.62	**.000**
Mid	5.15	F= 3.54	**.012**
Late	0.68	F= 22.48	**.000**
(C) 1-way ANOVA of period effect on release of water extractable phosphorus from plants:	*SS*	*Test Stat*	*Sig.*
Volunteer wheat	0.04	F= 8.36	**.002**
Hairy vetch	0.00	F= 1.92	.169
Cereal rye	0.05	F= 12.62	**.000**
Oat	0.53	F= 3.27	.056
Oilseed radish shoot*	38.94	F= 11.88	**.000**
Oilseed radish root	3.77	F= 5.21	**.013**
(D) 2-way ANOVA of period x CC plot (monocultures) effect on release of water extractable phosphorus from soil*:	*SS*	*Test Stat*	*Sig.*
Period	2.36	F= 2.15	.120
CC plot	13.09	F= 3.97	**.001**
Period x CC plot	7.67	F= 1.16	.314
(E) 2-way ANOVA of CC culture x period effect on release of water extractable phosphorus from cereal rye:	*SS*	*Test Stat*	*Sig.*
Period	0.07	F= 17.51	**.000**
CC culture	0.02	F= 7.89	**.007**
Period x CC culture	0.00	F= 0.88	.423
(F) 2-way ANOVA of CC culture x period effect on release of water extractable phosphorus from oilseed radish shoot:	*SS*	*Test Stat*	*Sig.*
Period	814.05	H= 3.29	.193
CC culture	987.01	H= 3.99	**.046**
Period x CC culture	4021.45	H= 16.25	**.000**
(G) 1-way ANOVA of period effect on release of water extractable phosphorus from oilseed radish shoot*:	*SS*	*Test Stat*	*Sig.*
Monoculture	38.94	F= 11.88	**.000**
Biculture	4.12	F= 1.76	.194
(H) 2-way ANOVA of CC culture x period effect on release of water extractable phosphorus from oilseed radish root*:	*SS*	*Test Stat*	*Sig.*
Period	8.19	F= 5.25	**.009**
Plot	2.66	F= 3.41	.072
Period x Plot	2.68	F= 1.72	.191

Table 4.2 Plant and soil properties of cover crop plots located in Bloomingdale, ON. from the 2016/17 non-growing season. All values shown are averages ± standard deviation, with the exception of plant total phosphorus concentrations from the October 12, 2016 sampling, which were not replicated. Plant biomass was sampled October 12, 2016, and soil bulk density was sampled in October 2017.

Cover Crop Plot	Soil Bulk Density (g/cm^3)	Plant Biomass (dry kg/ha)	Max Soil WEP (kg/ha) & Date Sampled	Plant TP Content (kg/ha)	Max Plant WEP (kg/ha) & Date Sampled
Control (volunteer wheat)	1.19 ± 0.09	1390 ± 251	1.10 ± 0.43 (Oct 17)	5.34	0.18 ± 0.05 (Nov 23)
Hairy Vetch	1.17 ± 0.05	1688 ± 238	3.14 ± 3.38 (Oct 17)	4.83	0.08 ± 0.02 (Oct 26)
Cereal Rye	1.22 ± 0.08	1872 ± 408	2.22 ± 0.89 (Oct 17)	7.06	0.17 ± 0.02 (Oct 12)
Oat	1.19 ± 0.10	4635 ± 385	3.46 ± 0.96 (Oct 17)	9.73	1.05 ± 0.20 (Oct 26)
Oilseed Radish Shoot	1.24 ± 0.08	4805 ± 1720	1.15 ± 0.02 (Dec 7)	10.94	0.62 ± 0.16 (Nov 23)
Oilseed Radish Root	1.24 ± 0.08	1234 ± 201	1.15 ± 0.02 (Dec 7)	3.85	1.98 ± 1.32 (Jan 18)
Biculture (rye and radish)	1.07 ± 0.03	2352 ± 1230	1.64 ± 0.47 (Mar 21)	7.29	0.46 ± 0.35 (Mar 21)
Polyculture*	1.14 ± 0.01	4540 ± 1440	1.61 ± 0.42 (Oct 17)	12.02	1.73 ± 1.31 (Nov 23)

*The polyculture included: cereal rye, oilseed radish, oat, hairy vetch, phacelia, pea, crimson clover, faba bean, sunflower, sorghum, and flax

The timing of changes in WEP pools also differed among species following trends in the regional weather variability (Figure 4.2). Although the three frost-tolerant species (cereal rye, hairy vetch, and volunteer wheat) all maintained relatively low WEP pools throughout the NGS in comparison to other CC species (Figure 4.3), WEP pools in both volunteer wheat and cereal rye declined significantly over the three periods following repeated exposure to FTC, whereas hairy vetch did not show a significant change in WEP pools (Table 4.1c). In contrast, the two frost-intolerant species, oat and oilseed radish, demonstrated different patterns in WEP availability over the NGS. For example, oat was strongly affected by the early autumn FTC (Figure 4.3), where median [WEP] increased by a factor of five

between the first and second sampling campaigns (no precipitation fell between these occasions). Following these early FTC, the oat CC was frost-killed and began to decompose over the remainder of the NGS. Oat WEP concentrations remained elevated compared to the frost-tolerant CC species (Figure 4.3), and did not differ significantly between the remaining periods of the NGS. Consequently, the effects of period on WEP content were not significant for oat (Table 4.1c). In contrast to oat, the WEP pool in oilseed radish differed significantly throughout the NGS in both shoot and root tissue (Table 4.1c). Similar to oat, the greatest WEP availability in the shoot tissue of oilseed radish were experienced in the early period, following light FTC (temperatures warmer than -10 °C). During the early period, WEP availability in the shoot tissue doubled after the plants were frost-killed, whereas the root tissue remained unaffected (Figure 4.3). However, WEP availability in the oilseed radish roots increased considerably during the mid period following heavy FTC (temperatures between -10 °C and -20 °C). Following this, WEP pools in the radish roots were smaller in subsequent sampling campaigns although they remained higher than the fall baseline.

Water extractable P concentrations were consistently less than 5% of the total vegetation P pool for hairy vetch, cereal rye, winter wheat, and oilseed radish shoot samples (Table 4.2). In contrast, WEP concentrations in oat (5-10%) and oilseed radish roots (>20%) were a greater proportion of total vegetation P. Although WEP availability varied in some species throughout the NGS, the total P (TP) content of the CC monocultures did not vary significantly over time, with the exception of oat, which declined throughout the NGS (data not shown).

4.4.3 Phosphorus Release from Soils and Phosphorus Concentrations in Runoff and Infiltrated Water

Median soil WEP pools in the top 5 cm of the monoculture plots typically ranged between 0.5 to 2 kg ha^{-1} (Figure 4.4), and maximums ranged from 1-3.5 kg ha^{-1} (Table 4.2). The soil WEP pool was typically greater than the vegetation WEP pool (Figures 3,4; Table 4.2), and only a few of the highest P contributing species (oilseed radish, the polyculture) released as much P as the lower end of the soil

WEP range (Table 4.2). Soil WEP concentrations and pools within the CC plots and the control plot were similar in magnitude. A two-way ANOVA testing the effects of CC plot and period on soil WEP pools found significant differences only among the CC plots ($F = 3.97$, $p = 0.001$; Table 4.1d). Greater soil WEP pools were observed in the oat, rye and vetch plots in comparison to the control and oilseed radish plots (not all data shown; some shown in Figure 4.4). It should be noted that the oat, rye and vetch plots were located at lower topographic positions in the field than the control and radish plots (Figure 4.1). Although soil WEP pools varied throughout the NGS, values did not differ significantly with Period (Table 4.1d) and they did not appear to relate to seasonal changes in plant WEP concentrations in the CC (Figures 3,4). Even the highly elevated peak in WEP availability in the oilseed radish root in mid-winter did not produce elevated WEP concentrations in the top 5 cm of soil, which remained low throughout the NGS (Figure 4.3, 4).

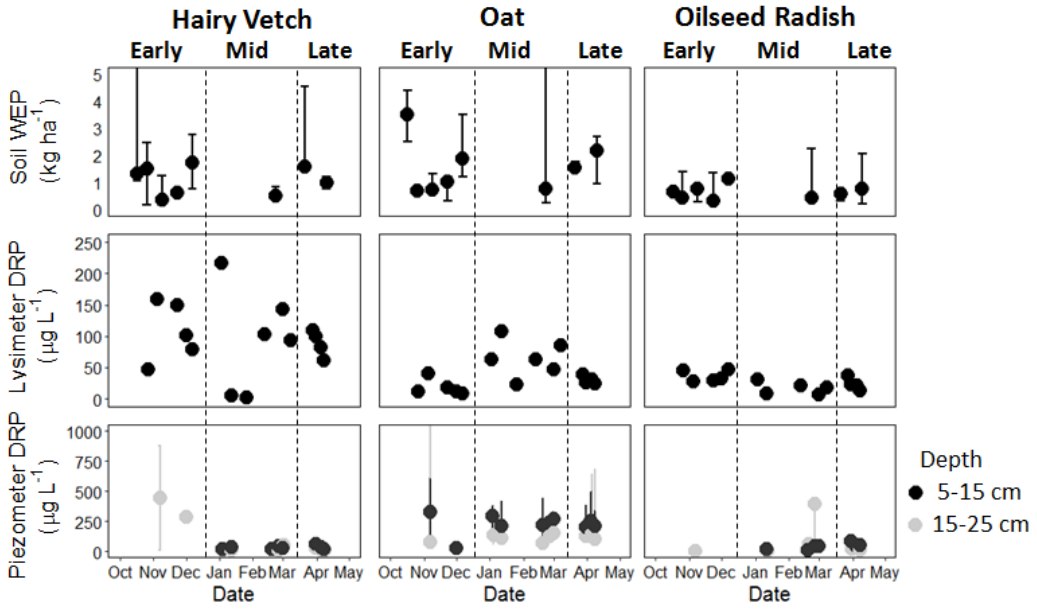

Figure 4.4 Median (point) and 95% confidence intervals (bars) for water extractable phosphorus (WEP) concentrations released from soils, and dissolved reactive phosphorus (DRP) concentrations from water collected in lysimeters and piezometers under plots of hairy vetch, oat, and oilseed radish. Sampling events have been split by periods of the non-growing season by weather conditions.

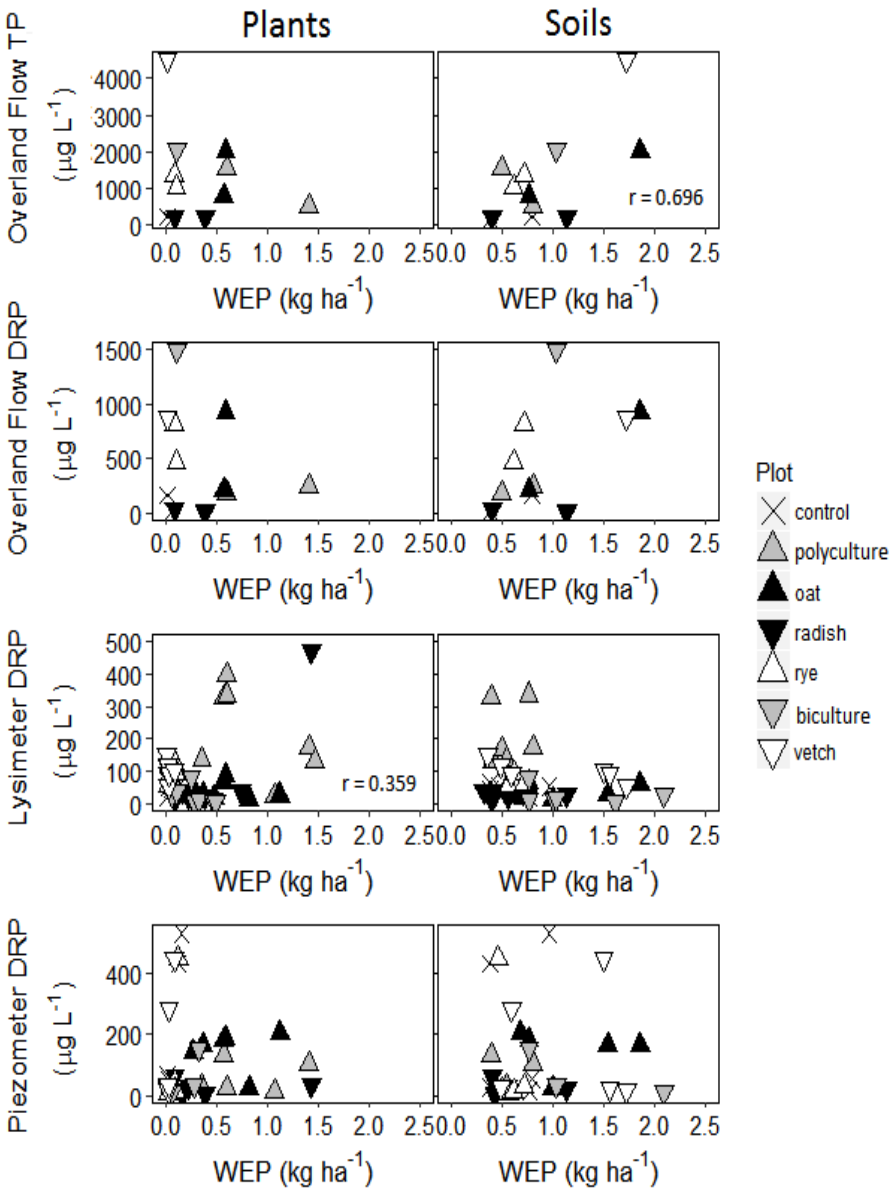

Figure 4.5 Median (point) concentrations of dissolved reactive phosphorus (DRP) from overland flow, lysimeter, and piezometer (both 5-15 cm and 15-25 cm depths combined) samples plotted against the average of water extractable phosphorus (WEP) pools extracted most recently before each water sample collection. Panels contrast WEP pools from plants (left) and soils (right). The r value was included in panels where a significant correlation (at an alpha value of 0.05) was observed.

Concentrations of DRP in leachate collected in the lysimeters were generally in the range of 0.01 - 0.3 mg L^{-1} (Figures 4,5). Although concentrations in leachate fluctuated throughout the study period, these changes did not coincide with observable changes in vegetation or soil WEP (Figure 4.3,4). There was some spatial variability in the field, where concentrations in leachate from the oilseed radish, oat,

rye, and control plots were similar (< 0.1 mg L^{-1}), whereas the lysimeters in the vetch and polyculture plots had greater concentrations (0.1-0.2 mg L^{-1} and 0.1-0.5 mg L^{-1}).

Water samples collected from piezometers (which held water moving through the soil matrix at various depths in the vadose zone) showed small baseline DRP concentrations comparable with DRP concentrations in lysimeter samples. Dissolved reactive P concentrations varied between the depths (5-15 cm versus 15-25 cm), with smaller concentrations occurring in the piezometer samples at lower depths (e.g. Figure 4.4). Concentrations of DRP in shallow groundwater exhibited a great deal of temporal variability, with the greatest concentrations occurring during the early period (Figure 4.4) during the initial wetting phase following a significant drought in 2016. The earliest piezometer samples were collected after a 22 mm rainfall event (Figure 4.2), the first precipitation event in over a month, and resulted in concentrations > 0.5 mg L^{-1} DRP in shallow groundwater, with several samples as high as 1-3 mg L^{-1}. While DRP concentrations were elevated in some plots later in the mid and late periods, no concentrations reached the initial range. Elevated concentrations observed within the various plots during the mid and late periods were not consistent in timing nor magnitude between the plots (Figure 4.4). The oat plot showed consistently elevated DRP concentrations at both depths throughout the study period (Figure 4.4).

Dissolved reactive P concentrations in lysimeter samples and shallow groundwater were compared with plant and soil WEP pools taken prior to the rain event to determine if DRP concentrations in runoff reflected either P source (Figure 4.5). Dissolved reactive P concentrations from lysimeters (all CC plots grouped together) were significantly correlated with plant WEP pools ($t = 2.33$, $df = 37$, $p = 0.025$), but the correlation was weak ($r = 0.359$). Surface soil WEP was not significantly correlated with lysimeter DRP, and neither potential source was significantly correlated with piezometer DRP.

Despite the large number of FTC and rain events over the NGS, overland flow seldom occurred during the study period (only on 4 occasions), and, overland flow did not occur consistently across the plots, primarily due to field topography. In several of the events that generated small amounts of overland flow, overland flow drained down the middle of the field along the point where the CC plots met (Figure 4.1), preventing the comparison of P concentrations among plots. Overland flow collectors were placed away from this zone to avoid the potential of runoff mixing from multiple CC plots, and consequently water did not reach all collection reservoirs during small events. Due to the infrequent nature of surface runoff generation, and topographic differences in the generation of surface runoff, water samples of overland flow are limited. This was particularly apparent in the hairy vetch and paired rye radish plots, located on the west side of the field which only produced 1 and 3 samples, respectively. Concentrations of P in overland flow varied considerably, with concentrations ranging from 0.025 - >1 mg DRP L^{-1} and 0.01 - 9 mg TP L^{-1}. It was hypothesized that following FTC, CC plots with elevated WEP pools prior to the thaw event would yield greater DRP concentrations in surface runoff. However, overland flow DRP concentrations were not significantly correlated with plant or soil WEP (Figure 4.5). It was also hypothesized that TP concentrations in overland flow would be greatest in control plots and smallest in plots with the greatest biomass. Concentrations of TP were generally the smallest in the control, oilseed radish, and mixture plots, which were located in the upslope topographic positions, while oat, rye, biculture and vetch, which were located lower down-slope had more elevated TP concentrations (Figure 4.5). No significant correlations were found between plant WEP concentrations and overland flow TP concentrations (Figure 4.5); however, TP concentrations were significantly correlated with surface soil WEP ($t = 3.06$, $df = 10$, $p = 0.012$) with a strong correlation of $r = 0.696$. In the control and radish plots TP was predominantly in the particulate form (>0.45 µm; based on ratios of DRP:TP) with DRP consisting 19% and 12% of TP, respectively (from the median of all concentration

ratios); whereas the oat, rye, and polyculture plots were more evenly split between DRP and particulate P, with DRP consisting of 48%, 48%, and 46% of TP, respectively.

4.4.4 Phosphorus Release from Cover Crops Planted in Biculture and Polyculture

Samples of cereal rye and oilseed radish grown in monoculture were compared with samples grown in a biculture to evaluate how the pairing might modify the impact of FTC on WEP pools from each subset (rye, radish shoot, and radish root) (Figure 4.6). A series of two-way non-parametric ANOVAs were done using CC plot (e.g., rye in monoculture or polyculture or radish shoot in monoculture or polyculture, etc.) and period of the NGS as factors. For cereal rye tissue, significant differences in plant WEP availability were found with both CC culture and period (Table 4.1e) and no interaction was observed among factors. Differences in WEP pools between cultures were driven by the larger rye biomass in the monoculture plot, compared to the smaller rye subset in the biculture (Table 4.2; subset data not shown). While WEP concentrations in both plots declined over the NGS, the monoculture plot had slightly greater WEP availability during portions of the early period (Figure 4.6). The ANOVA for oilseed radish shoot tissue demonstrated a significant interaction term between CC plot and period ($H = 16.25$, $p \leq 0.001$; Table 4.1f), therefore the data were into the groups of monoculture and polyculture samples, which were tested with a one-way ANOVA to evaluate the effect of NGS period (Table 4.1g). Samples from the monoculture plot exhibited a significant effect from the period of the NGS, as the WEP concentrations declined through the mid and late periods, whereas WEP concentrations from the biculture plants remained consistent, and were not significantly impacted. The effect of pairing oilseed radish root samples were found to be significantly affected by NGS period, with greater plant [WEP] observed later in the season, but this did not appear to differ between roots grown in monocultures or biculture (Table 4.1h).

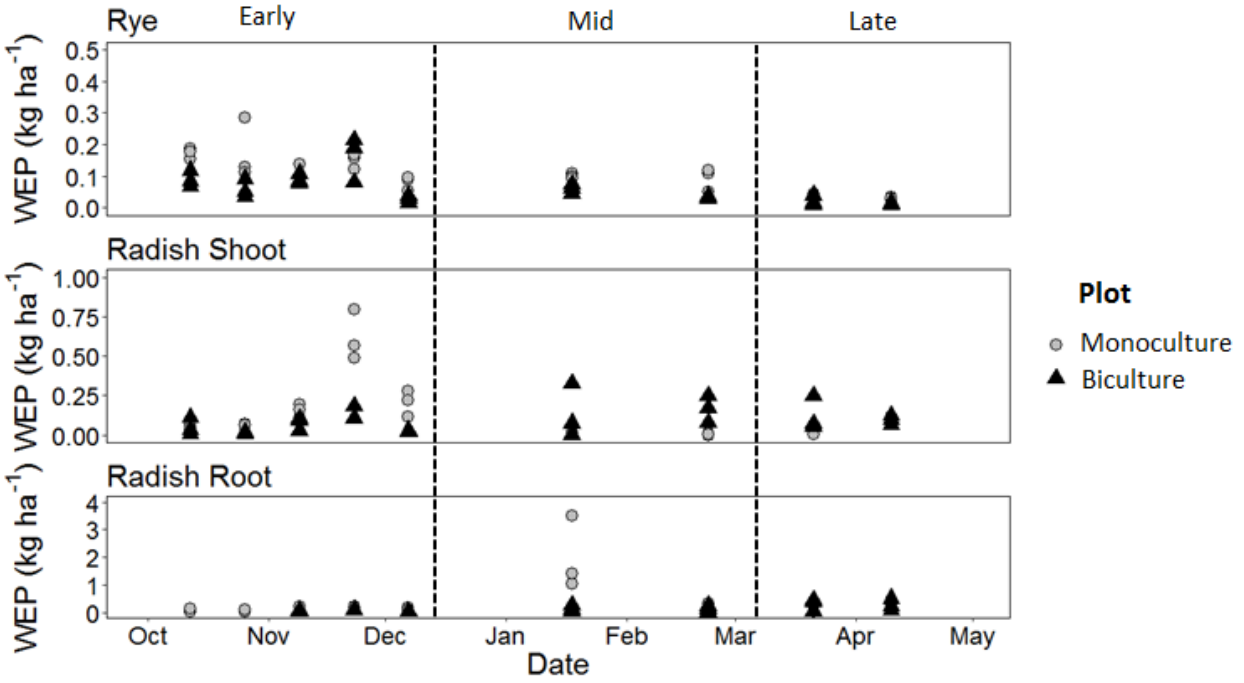

Figure 4.6 Pools of water extractable phosphorus (WEP) released from cereal rye and oilseed radish samples, contrasting plants grown separately in two monoculture plots, and together in one paired polyculture plot. Points show the WEP pool from each sample replicate. Samples were collected throughout the 2016/17 non-growing season, and have been split into three periods by weather conditions. No samples of radish root were collected from the biculture in October; otherwise WEP ranges overlap where only one symbol appears.

Figure 4.7 illustrates the breakdown of WEP concentrations from the mix of 11 species, broken into the groupings of grasses, legumes, non-legume broadleaves, and oilseed radish root. As was found in the comparison of rye and radish grown as monoculture and polyculture, species included in the 11-species mix demonstrated similar responses to FTC exposure to samples grown in monoculture. Indeed, concentration ranges from the legumes were small, similar to hairy vetch, whereas WEP concentrations from grasses and non-legume broadleaves were greater, indicating similar trends of P availability compared to oat and oilseed radish shoots (Figures 3, 7). It was hypothesized that when plants were grown in polycultures, P release from tender plants could be taken up by hardier species, thereby reducing P in runoff. However, the greatest DRP concentrations in soil water collected in lysimeters were consistently found in the mix plot (Figure 4.5).

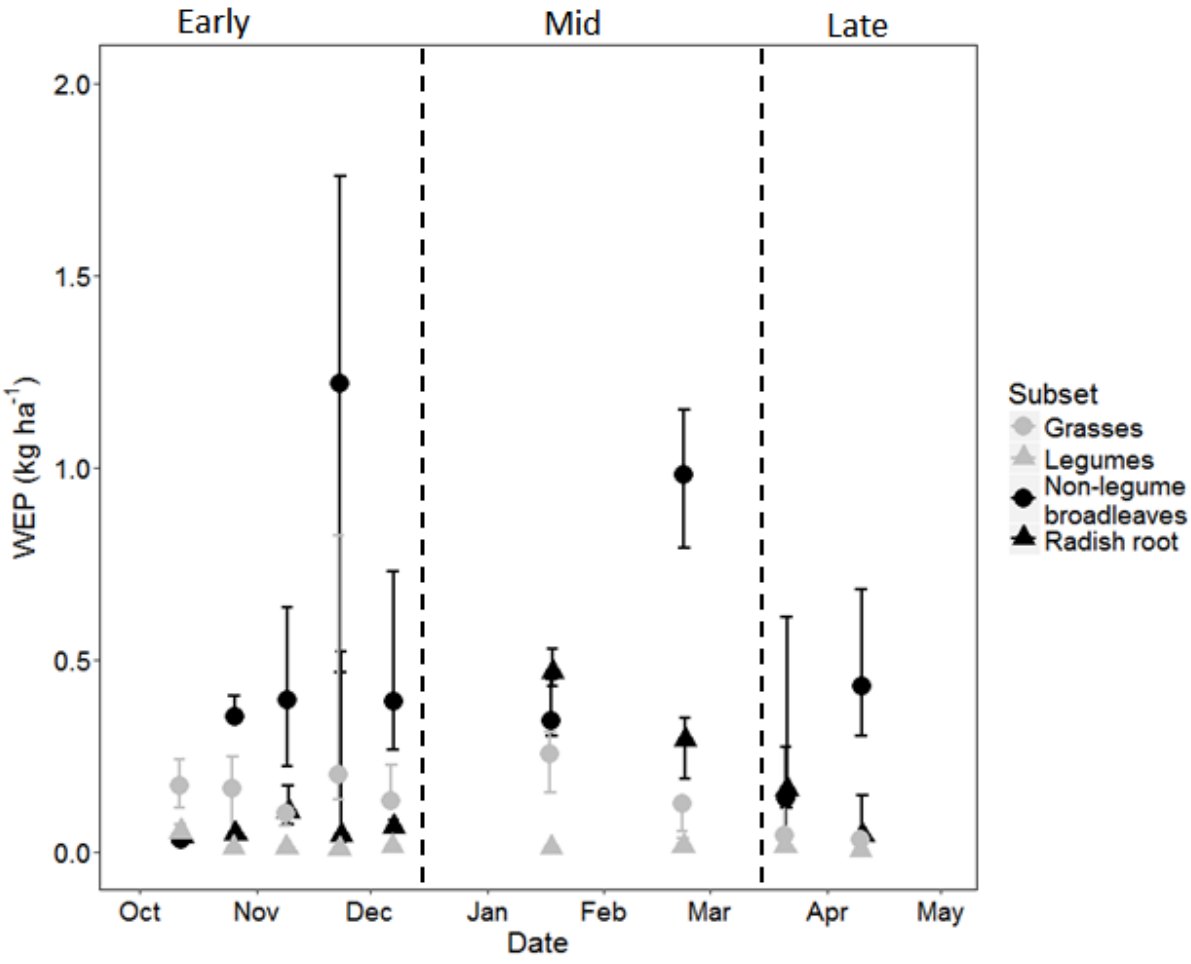

Figure 4.7 Median (point) and 95% confidence intervals (bars) for water extractable phosphorus (WEP) pools in cover crops planted in an eleven species polyculture, and collected from a field throughout the 2016/17 non-growing season. Cover crops have been grouped together into subsets based on taxonomic similarity. Sampling events have been split by periods of the non-growing season by weather conditions.

4.5 Discussion

4.5.1 Differences in Phosphorus release among Cover Crop Species

The observed differences in P release between CC species were consistent with previous work that suggests that some species may be more susceptible to the impacts of FTC than others (Miller et al., 1994; Sturite et al., 2007; Liu et al., 2014; Øgaard, 2015; Lozier and Macrae, 2017; Cober et al., 2018). In the current study, field observations of smaller WEP concentrations from more frost-tolerant species (cereal rye and hairy vetch) and larger WEP concentrations from frost intolerant species (e.g. oat, radish)

are consistent our previous lab studies (Cober et al., 2018). In the case of the frost-tolerant species, the lack of P release in response to FTC may be a result of several mechanisms. It is possible that frost-tolerant CC species translocate a large proportion of P that would otherwise be water extractable, storing it in tissues that are less susceptible to leaching (Chapin, 1980). Plants have also been found to modify the fractionation of P over the NGS, with larger fractions of organic P dominating during the winter months, eventually being converted to inorganic P again in the spring (Chapin and Kedrowski, 1983). Morton (1977) describes a pattern of P translocation from the leaves of a perennial grass in a mild winter climate. Much of the existing research on mechanisms of P retention in plants has been done on non-agricultural species in colder regions: thus, it is difficult to identify the precise mechanisms used by these CC species. However, the low P release, and decline in P availability in response to early NGS FTC suggests that frost-tolerant CC may be better options managing P release in cold regions.

For the frost-intolerant species, the potential for P release following FTC has been identified previously. Numerous laboratory studies have demonstrated great potential for P leaching from oilseed radish (Miller et al., 1994; Liu et al., 2014; Øgaard, 2015; Cober et al., 2018), or oat (Cober et al., 2018; Lozier and Macrae, 2017), which is consistent with the significant increase in WEP pools from oilseed radish and oat in response to the FTC conditions of this field study (Table 4.1b; Figure 4.2). However, it is important to note that the magnitude of WEP availability from these species is much smaller (3x less) in the field setting compared to what has been observed in the laboratory (e.g. Cober et al., 2018). This response difference is likely due to the senescence of the oat tissue that occurred within a week of the first killing frost, which Cober et al. (2018) noted did not occur in the lab experiment. Similar to oat, Elliot (2013) found that living winter wheat released significantly more P than dried wheat stubble. It is likely that the drying of the oat shoots reduced the immediate availability of WEP, and the gradual decomposition with further exposure to FTC resulted in the consistent decline of TP.

The high concentration of WEP released from oilseed radish and oat samples through the mid and later periods of the NGS (Figure 4.3) suggests that the species may be poor choices for CC users in areas where P loss is of concern, particularly in fields where overland flow and ponding occur regularly. The great concentration of WEP in plant tissues (greater than 1100 mg/kg), presents a great risk for P release given the sizeable biomass of oilseed radish produced (Table 4.2). Indeed, with only emergent portions of root sampled for biomass, the radish root has even greater potential to act as a P source. However, despite the high WEP concentrations leached from oilseed radish shoots and roots during the mid period, the concentration of TP in both subsets did not decline over time.

The size of CC WEP pools were assessed with a shaking extraction, a common practice for evaluating potential nutrient loss (Roberson et al., 2007; Øgaard, 2015; Lozier and Macrae, 2017; Cober et al., 2018). Shaking extractions may result in higher leached P concentrations than rainfall would be able to remove from samples and are not a direct analog for natural leaching (Lozier and Macrae, 2017). However, the degree of P leaching in natural systems may occur via different modes of action, for example, water ponding on saturated soils may leach greater concentrations of P from CC than rain or shaker extractions, such that shaking plant samples certainly captured some of the natural range (Lozier and Macrae, 2017). For P export to occur, a strong leaching mechanism must be concurrent with hydrological transport.

4.5.2 Relationships between the Cover Crop Phosphorus Pool, Soil Phosphorus Supply, and Runoff Phosphorus

The release of P by CC was not apparent in soil pools following runoff or potential leaching events. Indeed, the differences in WEP concentration found between CC sampling events were so minimal that any impact of leached CC P was not apparent within the natural variability of the soil P concentrations. While there was inherent and significant variability in soil WEP concentrations between the plots ($F = 3.97$, $p = 0.001$), the range of soil WEP content was similar between the control and

several CC plots before and during exposure to FTC. As well, some of the variability in soil P concentrations may be related to the topography of the field (e.g. greater soil P in the oat and hairy vetch plots, which were located in the down slope topographic position, Figure 4.1), as trends have been shown with greater soil P occurring at the bottom of hill slopes (Moore et al., 1993; Kozar et al., 2002).

The results of this study suggest that the differences in P content in water samples may be more strongly related to legacy P in the soil than leached plant P. The low concentrations of P in lysimeter samples (Figure 4.4) suggest that either very little P was leached from the CC, the movement through the soil matrix enabled adsorption to the soil, or both. The significant correlation between plant WEP and lysimeter DRP ($t = 2.33$, $df = 37$, $p = 0.025$, $R = 0.359$), and not soil WEP, is indicative of the short contact time infiltrating water had with the top 15 cm of the soil column before settling in the lysimeter reservoir. This limited contact time may have been in part due to the presence of a well-developed macropore network. Indeed, many CC roots penetrated fully through the soil block to the opening of the reservoir due to the fact that the lysimeters only captured the top 15 cm of soil. The presence of these macropore networks has been shown to limit P sorption, and there is concern that macropore networks may lead to increased P loss through tile drains (Heathwaite and Dils, 2000; Geohring et al., 2001; Beven and Germann, 2013). However, tile depth in Southern Ontario is typically 60 to 90 cm (*e.g.* Van Esbroeck et al., 2017), which means lysimeter concentrations observed in this study are not directly comparable to potential edge of field loss from tiles.

The smaller DRP concentrations in the deeper piezometers (15 to 25 cm) relative to shallower piezometers (5 to 15 cm) indicate that P release from plants or surface soils will largely be buffered during infiltration into the deeper soil profile. This is likely due to the strong sorption affinity of mineral subsoils (Holtan et al., 1988). As expected, the greater soil residence time of by piezometer water samples produced no significant correlation with plant or surface soil WEP. Riddle and Bergstrom (2013)

found that soil cores were able to reduce P leaching from CC by up to 98%, with variability between soil textures and CC species; which agrees with these results after reasonable soil residence times. Given the high value for soil test P from this field site (26.5 +/- 8.3 mg kg^{-1}), there is potential for other fields with lower soil test P to exhibit even greater buffering of P in leachate.

In addition to the legacy P pool, another possible source of P in the vadose zone may be soil microbes impacted by FTC (Schimel and Clein, 1996; Larsen et al., 2007). Just as with plant tissue, exposure to FTC can lyse microbes resulting in a significant flush of DRP within the soil. Schimel and Clein (1996) suggest a single freezing event may kill up to 50% of the microbial community. The combination of snowpack and CC provided an insulating layer for much of the field, and prevented freezing ambient temperatures from penetrating into the soil for most of the NGS (Figure 4.2). However, the magnitude and number of FTC occurrences was greater in the control plot and in the oilseed radish plot, which had little remaining plant cover by January (data not shown). Both plots had light frosts (-2 °C) penetrate to depths of 5 cm that did not occur elsewhere in the field, which could have resulted in the slightly higher DRP concentrations observed in the upper piezometer water samples during the late period (Figure 4.4).

The occurrence of extremely high TP concentrations in overland flow samples (e.g. 1 to 2 mg L^{-}) agrees with literature, which has shown the potential for overland flow to move large amounts of P directly off of fields (Sharpley and Kleinman, 1998; McDowell et al., 2001; Macrae et al., 2007; Gentry et al., 2007). The ratio of DRP to TP was greater than the control in all CC plots except oilseed radish, likely a combination of increased DRP in other plots and a reduction in PP loss. The greater PP concentrations were related to the degree of ground cover during the mid period. As the control and oilseed radish plots had the least residue cover during the mid period (no CC planted and rapid decomposition,

respectively), the soils were exposed to forces that increased PP export (Sharpley and Kleinman, 1998; Sharpley and Smith, 1991).

While some contribution of DRP from CC leachate should be expected, no consistent trend was observed among the different CC plots. For example, while the radish root had a much greater WEP pool than oat (Figure 4.3), the oat plot had more DRP in overland flow than the radish plot (Figure 4.5). In contrast, the greater TP concentrations in surface runoff from the rye plot show no contribution from the plant leaching (in which agrees with the poor correlations with plant WEP), but suggests a stronger contribution from soil P. The lack of the expected trend between runoff P and CC WEP may be due to temporal differences in P supply and transport timing, as different CC species have been shown to release P under different FTC conditions (Øgaard, 2015). For example, the greatest oilseed radish WEP pool was observed after the first overland flow event occurred had occurred, and oilseed radish WEP declined by the second overland flow event. Similarly, Roberson et al (2007) expected to see a significant increase in runoff P concentrations from CC contributions, but suggested the natural variability in the field system, FTC timing, and precipitation may have reduced the potential for P export from the field.

More field trials are needed to better quantify the potential impact of overland flow on P loss from CC, which should consider the impact of different soils, variation in soil test P, and the impact of slope. This work was limited in scope to analysing water concentrations, and future studies should consider developing a more complex flow weighted mean P concentration, to determine the full extent of CC P field loss. As well, despite the potential for overland flow to move DRP, environmental managers should consider the other beneficial properties of CC (Kaspar et al., 2001; Kleinman et al., 2005; Kaspar and Singer, 2011).

4.5.3 Impacts of Mixing Cover Crops on Phosphorus Loss

The polyculture showed no potential benefit of including frost-tolerant species on P leaching from frost-intolerant CC. Indeed, the group of non-legume broadleaves showed the potential to release the greatest concentrations of P (within the same range as the P pool in surface soils). Combinations of CC which only contain high risk species may release more P compared to the same monocultures because of overyielding—the capacity for greater primary productivity in a diverse mixture than a single species—however, the actual extent of this effect is not fully understood (Cardinale et al., 2011; Wortman et al., 2012; Cardinale et al., 2013). However, while the large biomass which developed in the polyculture (Table 4.2) certainly contributed to the greater WEP pool, the large WEP concentrations from frost-intolerant species remained a driving factor. The low WEP concentrations from the legumes group (Figure 4.7), has demonstrated the potential for CC polycultures to be grown without greater risk of P loss. For example, the average biomass of oilseed radish root and the legumes was equivalent, but the WEP pools differed immensely. By choosing specific species with little WEP release in CC mixtures, such as hairy vetch, cereal rye, or red clover (Cober et al., 2018), growers may still be able to utilize multiple other CC benefits while limiting the risk of P leaching. Myers and Watts (2015) suggest approximately 25% of CC users in the United States plant mixtures (which is expected continue to increase); despite this, Chu et al. (2017) suggest the current literature on CC mixing is limited, and its efficacy is uncertain. Indeed, literature on the impacts of mixing CC on P loss is less available, and more research is needed to better evaluate this increasingly popular practice.

The biculture of cereal rye and oilseed radish demonstrated that the effect of FTC on the temporal trend of WEP release may be altered in mixtures. Comparisons were done based on field scale P pools, which influenced in part by sizeable differences in plot biomass (Table 4.2). However, differences in plant WEP concentrations demonstrate the same pattern of temporal trends, such as in the radish shoot, which had median concentrations of 0.5 mg kg^{-1} extracted from the monoculture, and

180 mg kg^{-1} from the biculture (which was consistent through the rest of the NGS). By limiting the exposure of extremely low temperatures during the mid season, the biculture prevented a singular burst of P release from the oilseed radish (likely after the first heavy FTC), which would be harder for soils to capture compared to a slower release. Limiting the potential for these hot moments of P loss, planting a pairing of oilseed radish with a frost-tolerant species may be a better option than oilseed radish monocultures for farmers that want the benefits of a radish crop, such as compaction reduction.

4.6 Conclusions

When exposed to climate conditions similar to those of this field season (Figure 4.2), CC are not a greater source of P export than natural sources in agricultural fields, including the soil P pool. Some species of CC exhibited the potential for greater WEP release at various points of the field season. Frost-tolerant species indicate better potential for limiting the impact of plant P release, whereas frost-intolerant CC species such as oat and oilseed radish demonstrated potential for higher P release at various points during the NGS. The extent of P leaching and export will depend on regional climate conditions, such as the timing and intensity of FTC relative to precipitation, as well as field characteristics, such as soil texture, slope, soil test P, and the occurrence of soil frost.

Natural variability in P loss from agricultural systems made quantifying the impact of CC difficult; compared to the control, plots with CC had similar concentrations of soil P, as well as DRP in infiltrated water. The occurrence of overland flow indicates potential for P loss from CC; however, P in surface soils may have a larger contribution to overland flow TP concentrations than most CC. Growing CC in polyculture shows the potential for cold-tolerant species to insulate frost-intolerant species against the impact of FTC, and may alter the pattern of WEP release, but more research is needed.

Planting CC should remain an agricultural BMP in the Great Lakes region. Species choice should be dependent on site specific characteristics and climate; however, frost-tolerant species are

recommended for colder regions where P loss is a concern. More plot and field scale studies are needed to evaluate the efficacy of CC in other regions where CC may be exposed to more intensive freezing and consistently frozen soils. Future studies should sample CC tissues frequently to avoid missing potential P release events and should specifically consider the impact of overland flow on CC P export from fields.

Chapter 5- Major Conclusions of Thesis

The potential for a CC to act as a source of P after exposure to FTC has been well demonstrated in the literature (Miller et al., 1994; Sturite et al., 2007; Bechmann et al., 2005; Riddle and Bergstrom, 2013; Liu et al., 2013; Liu et al., 2014; Lozier and Macrae, 2017; Øgaard, 2015;). This thesis has examined several factors that affect the degree of P leaching from CC both in the laboratory (Chapter 3) and field (Chapter 4). The laboratory experiment described in Chapter 3 demonstrated that the magnitude of FTC can have a significant impact on the concentration of P leached from CC tissues. Minimal FTC exposure, at temperatures of -4 °C to 4 °C did not significantly elevate P concentrations, whereas heavy FTC at -18 to 4 °C and -18 to 10 °C significantly increased extracted P concentrations. The effect of heavy frost was expected compared to results from Bechmann et al. (2005), Liu et al. (2013), Liu et al. (2014), and Miller et al. (1994), who all observed similar increases in P. The magnitude of FTC did not impact the release of ammonium and nitrate, which was consistent with the findings of Miller et al. (1994). The lack of impact of minimal frost on P loss from CC species in the lab experiment was consistent with what was also observed in the field experiment (Chapter 4). Indeed, the field study demonstrated that P extraction from several CC species was not affected by the FTC experienced in the Southern Ontario climate.

This thesis has also shown that P release varies between CC species in response to the same FTC exposure. The results of the lab study demonstrated a hierarchy among the chosen CC species for potential WEP release, with a wide range in P concentrations between hairy vetch, with the smallest WEP concentrations, to oilseed radish, with the greatest WEP concentrations from shoot and root tissues. Cover crop species which are frost-tolerant (hairy vetch, red clover, cereal rye) all showed relatively low potential for P loss, whereas CC species which winter-kill (oat, oilseed radish) demonstrated significantly greater potential for P loss. The results of the field study indicated similarly low potential for frost-tolerant CC species to act as a P source through the NGS, as hairy vetch, cereal rye, and the volunteer winter wheat all had low WEP concentrations, and cereal rye and volunteer

wheat showed a decline in concentrations in response to FTC. In cold regions where P export is of concern for freshwater eutrophication, specific CC species, such as hairy vetch, red clover, and cereal rye should be grown to mitigate the risk of P leaching. While a limited number of species were tested in this thesis, it is likely that this relationship between frost-tolerance and minimal P release should extend to other CC species. In regions where winters are warmer and FTC remain closer to 0 °C, or where consistent snowpack insulates CC over the NGS, more options are available to CC growers without additional risk for P leaching. Cover crop decision making tools and recommendations should include P leaching risk when balancing agronomic requirements and benefits of regionally available CC options.

This thesis has also demonstrated that herbicide induced termination of CC can significantly increase the concentration of extractable P similarly to CC exposed to heavy FTC. The results of the lab study show that termination did not have an additive effect in combination with heavy FTC, which was similar to results found by Lozier and Macrae (2017) who analysed the impact of FTC on terminated red clover (however, without comparison to living samples). Therefore, it may be optimal for CC users who choose to grow species that present a risk for greater P leaching to terminate crops in autumn. Autumn termination may enable P released from CC to infiltrate and be held in soils, whereas potential exists for greater P export in overland flow over frozen or saturated soils. However, frost-tolerant species such as should not be terminated until spring, as more P leaching may occur than would otherwise.

This thesis has also demonstrated that any potential P release from CC did not appear to affect soil and shallow groundwater P concentrations in the field and year of study. The results of Chapter 4 show that WEP leached from plants was typically small in comparison to the WEP pool in the surface soils (top 5 cm). Concentrations of DRP in infiltrate and shallow groundwater were generally low, with occasionally peaks; however, peak DRP concentrations were unrelated to the timing of potential CC P leaching, and generally did not occur in plots with the greatest available CC P. Comparable to this field

study, Roberson et al. (2007) also found no influence of P leached from CC in a field study, and suggest that the natural variability in soil and runoff P concentrations was a greater factor. In Southern Ontario, mild winter temperatures and snowpack mean CC are typically only exposed to mild FTC, such that P leaching from CC is likely to be minimal, and infiltration into unfrozen soils can hold leached plant P regardless.

This study has shown that growing CC in mixtures can influence P loss from CC. The results of Chapter 4 demonstrate that a biculture of cereal rye and oilseed radish had a difference in the timing of WEP release compared to monoculture radish, that may have resulted from insulation from the rye CC. Growing a polyculture of frost-tolerant and intolerant CC together was ineffective at limiting P loss. While growing mixtures of frost-intolerant CC may potentially contribute high P for leaching, growing mixes of frost-tolerant CC should limit P loss while providing additional agronomic benefits.

In conclusion, this thesis has demonstrated that the use of CC as an agricultural management practice should not be considered a risk for P loss if appropriate species are chosen for the specific climate conditions of a region. In areas with mostly mild FTC exposure, such as Southern Ontario, using frost tolerant CC species and waiting until spring for termination should be considered a BMP to reduce the risk for P leaching. Other regional factors, such as soil texture, and local topography may influence P loss and should be considered in addition to FTC temperatures. Future research should continue to evaluate the potential for P loss at the field scale; this thesis only examined one field season and one field site, and other climate conditions could affect CC P loss differently. As well, additional research into a wider variety of CC mixtures could help better inform farmers for making planting decisions.

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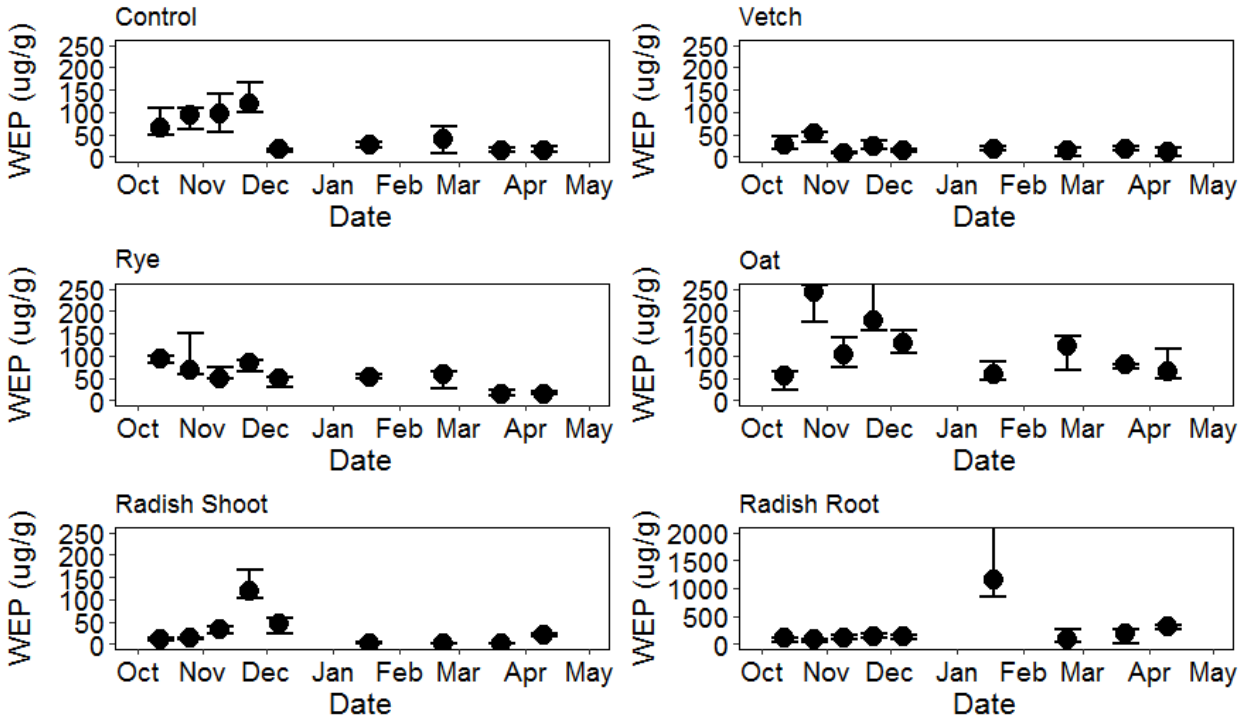
Appendix A



Median (point) and 95% confidence intervals (bars) for water extractable phosphorus (WEP) concentrations from cover crop samples of various species collected from a field site throughout the 2016/17 non-growing season. Note the different scaling used for oilseed radish root